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Electronic tags and genetics explore variation in migrating steelhead kelts (*Oncorhynchus mykiss*), Ninilchik River, Alaska

Jennifer L. Nielsen, Sara M. Turner, and Christian E. Zimmerman

Abstract: Acoustic and archival tags examined freshwater and marine migrations of postspawn steelhead kelts (*Oncorhynchus mykiss*) in the Ninilchik River, Alaska, USA. Postspawn steelhead were captured at a weir in 2002–2005. Scale analysis indicated multiple migratory life histories and spawning behaviors. Acoustic tags were implanted in 99 kelts (2002–2003), and an array of acoustic receivers calculated the average speed of outmigration, timing of saltwater entry, and duration of residency in the vicinity of the river mouth. Ocean migration data were recovered from two archival tags implanted in kelts in 2004 (one male and one female). Archival tags documented seasonal differences in maximum depth and behavior with both fish spending 97% of time at sea <6 m depth (day and night). All study fish were double tagged with passive integrated transponder (PIT) tags implanted in the body cavity. Less than 4% of PIT tags were retained in postspawn steelhead. Molecular genetics demonstrated no significant differences in genetic population structure across years or among spawning life history types, suggesting a genetically panmictic population with highly diverse life history characteristics in the Ninilchik River.

Résumé : Des étiquettes acoustiques et des étiquettes à archivage nous ont permis de suivre les migrations en eau douce et en mer de bécards de la truite arc-en-ciel anadrome (*Oncorhynchus mykiss*) après la fraie dans la rivière Ninilchik, Alaska, É.-U. Les truites arc-en-ciel après la fraie ont été capturées à un déversoir en 2002–2005. Une analyse hiérarchique révèle l'existence de multiples cycles biologiques migratoires et comportements reproducteurs. Nous avons muni 99 bécards d'étiquettes acoustiques (2002–2003) et un réseau de récepteurs acoustiques a permis de calculer la vitesse de migration vers la mer, le calendrier de l'entrée en eau salée et la durée de résidence dans les environs de l'embouchure de la rivière. Les données de migration en mer ont été obtenues de deux étiquettes à archivage fixées à des bécards en 2004 (un mâle et une femelle). Les étiquettes à archivage ont relevé des différences saisonnières dans la profondeur maximale et le comportement; les deux poissons ont passé 97 % de leur temps en mer à une profondeur <6 m (jour et nuit). Tous les poissons utilisés dans l'étude portaient aussi une étiquette PIT (transpondeur intégré passif) implantée dans la cavité corporelle. Moins de 4 % des étiquette PIT ont été retenues chez les bécards après la fraie. Une analyse génétique moléculaire ne montre aucune différence significative de structure génétique de la population, ni d'une année à l'autre, ni en fonction des types de cycle biologique reproducteur, ce qui laisse croire à l'existence dans la Ninilchik d'une population génétiquement panmictique possédant des caractéristiques hautement diversifiées de cycle biologique.

[Traduit par la Rédaction]

Introduction

Steelhead, the anadromous form of the Pacific salmonid species *Oncorhynchus mykiss* (Smith and Stearley 1989), originate from coastal streams and rivers throughout the North Pacific Ocean (Behnke 1966; Scott and Crossman 1973; Burgner et al. 1992). Their native anadromous range currently extends from southern California to the Kamchatka Peninsula (Quinn 2005). *Oncorhynchus mykiss* are iteroparous and can display partial anadromy with some populations maturing in fresh water (Jonsson and Jonsson 1993; Keefer et al. 2008; Narum et al. 2008).

Postspawning migrations of steelhead are typically dominated by first time kelts returning to the sea after their first spawning event, but can include older multisea winter adults who have spawned more than once. Among Pacific salmon, iteroparity is unique to steelhead and coastal cutthroat trout (*Oncorhynchus clarkii clarkii*), but is common in Atlantic salmon (*Salmo salar*). Migrations to and from salt water are energy demanding and physiologically challenging (McKeown 1984; Hendry and Berg 1999; Cooke et al. 2006). Despite the increased costs associated with secondary migrations, iteroparous fish are thought to contribute substantially to the genetic and demographic structure of some salmon populations (Ward and Slaney 1988; Fleming and Reynolds 2004; Keefer et al. 2008). Rates of iteroparity in steelhead vary widely (0%–79%) and can change considerably year to year (Withler 1966; Savvaitova et al. 1996; Narum et al. 2008). Repeat spawning is thought to be more common in females (Jones 1973; Burgner et al. 1992; Wertheimer and Evans 2005), suggesting valuable genetic trade-

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J.L. Nielsen,[1] S.M. Turner, and C.E. Zimmerman. US Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, AK 99508, USA.

[1]Corresponding author (e-mail: jlnielsen@usgs.gov).

offs from this life history (Keefer et al. 2008). We assume that there is an age-limited senescence to the number of times any individual can return to fresh water to spawn (Hendry et al. 2004), but data on long-lived steelhead are few. Jones (1973) documented one female steelhead in Petersburg Creek, Alaska, that had spawned five times; Leider et al. (1990) documented individuals spawning up to four times; and a male steelhead spawning for the sixth time was found on the South Fork Eel River, California (J.L. Nielsen, unpublished data). The length of time at sea between spawning events can also vary among steelhead, with adults spawning on consecutive or alternate years (Burgner et al. 1992; Lohr and Bryant 1999). These latter categories of steelhead life histories remain poorly described. Radio telemetry has been successfully used to describe migratory behavior of many salmonids in fresh water (Eiler 1995; Meka et al. 2003; Cooke et al. 2005). Ruggerone et al. (1990) used ultrasonic tags to individually track adult steelhead captured in British Columbia, as they migrated from the ocean to spawning locations in the Dean River. While we know something about the upstream spawning migrations of steelhead from radio telemetry studies, little is known about the behavior of postspawn steelhead during their downstream and saltwater migrations. Because of radio signal attenuation in salt water, this method is not generally applicable to migrations in marine habitats, although ultrasonic telemetry has been used to track salmon during their homeward migration at sea in near-surface waters (Ogura and Ishida 1992).

In recent years, acoustic technology has been developed and successfully applied for tracking the freshwater to marine transitions in anadromous fish (Voegeli et al. 1998; Welch et al. 2004; Kristianson and Welch 2007) and to monitor salmon in marine habitats (Voegeli et al. 1998; Lacroix and Voegeli 2000; Welch et al. 2004). The development of automated submersible receivers that can be deployed at key locations or in a gate-like configuration across constrictions or shallow marine areas has enabled the tracking of larger numbers of fish carrying coded acoustic tags (Welch et al. 2002; Heupel et al. 2006). While acoustic tags have been used to describe movements of juvenile salmon (Welch et al. 2004; Melnychuk et al. 2007), no evidence is found in the literature on the freshwater–marine migration of steelhead kelts (however, see Halttunen et al. 2009 for acoustic data on Atlantic salmon kelts).

Once steelhead enter open marine habitats, tracking their movements becomes more difficult. During their first year at sea, steelhead are thought to move rapidly offshore avoiding habitats along the continental shelf (Hartt and Dell 1986; Quinn 2005). Published high-seas research data suggest that young steelhead are widely distributed in offshore waters and that at least some adult steelhead from North America migrate across the Pacific Ocean into the western subarctic gyre off the Kamchatka Peninsula (Burgner et al. 1992; Myers et al. 1996; Myers et al. 2001). Despite over 80 years of research, we know little about the behavior of salmon during open-ocean migrations (Walker and Myers 2009).

Electronic data storage tags (DSTs, or archival tags) have been applied in many studies of fish distribution and movements in marine habitats (Metcalfe and Arnold 1997; reviewed in Sibert and Nielsen 2001). Many types of environmental sensors have been developed, but depth (pressure) and temperature are the most common sensors used in DST studies. This new technology holds great promise for understanding the oceanic distribution and behavior of different fish (Walker et al. 2000; Nielsen et al. 2009).

In this study, we used acoustic tags with moored receivers and long-term archival tags to explore the transit time of downstream migration, timing and duration of transition from fresh water to salt water, behavioral response to tides at first ocean entry, and environmental conditions during open-ocean migrations for postspawn steelhead kelts from the Ninilchik River, Alaska. We compiled life history and genetic data on downstream migrating fish at the weir and explored the variety of life histories found in steelhead kelts in this system. Population genetic analyses were used to explore genetic relationships among and between sample years and life history types of steelhead in the Ninilchik River. Our objective was to better describe the migratory and genetic characteristics of steelhead near the northern extent of their range and test the utility of electronic tags as tools to better understand the marine life stages of adult steelhead.

Materials and methods

Demographics

The Ninilchik River is a 350 km^2 watershed located on the southern Kenai Peninsula in south-central Alaska, USA (Fig. 1). The Ninilchik River flows into Cook Inlet, a large marine watershed (~100 000 km^2) that is a semi-enclosed coastal body of water connected to the North Pacific Ocean. Lower Cook Inlet is extremely rugged with deep pockets and shallow shoals (Fig. 1). The depths in the central inlet near the Ninilchik River are generally less than 60 m. Cook Inlet has the fourth largest tidal bore in the world (9.2 m). At the mouth of the Ninilchik River, the spring mean tidal range is 5.8 m, resulting in strong tidal currents during kelt outmigration. All downstream migrating steelhead adults were collected at a weir located at river kilometre 6 on the Ninilchik River (2002–2005; Fig. 2). The weir was installed as early as weather would allow in May and operated 24 h a day until the end of June 2002–2005, encompassing the full distribution of downstream migrating steelhead kelts (Table 1). After completion of our kelt study, the weir was operated as a full downstream blockage in an effort to monitor upstream migrating Chinook salmon (*Oncorhynchus tshawytscha*) by the Alaska Department of Fish and Game. No adult steelhead were observed at the weir following our study in any year the weir was operated.

Randomly selected individuals in good health (N = 162) were selected for tagging with electronic devices (2002–2003). No secondary external markers were used because of regulations imposed by Alaska Department of Fish and Game. All electronically tagged fish were implanted with individually coded passive integrated transponder (PIT) tags. PIT identifications were scanned for all fish passing the weir using a handheld detector. A metal detector was also used to scan all steelhead passing the weir (2003–2005) to monitor previously tagged fish that may have lost their PIT tag. Scales from the preferred area (Koo 1962) were taken from 160 tagged fish and seven freshwater adult males collected at the weir (Table 2). Scale patterns were used to identify reproductive life history types when annuli and spawning

Fig. 1. Map of Cook Inlet, Alaska, showing bathymetry and currents. Red circle is approximate location of mouth of Ninilchik River. (Graphic adapted with permission, G.S. Drew, originator: http://alaska.usgs.gov/science/biology/seabirds_foragefish/maps/Cook_Inlet/bathy.php.)

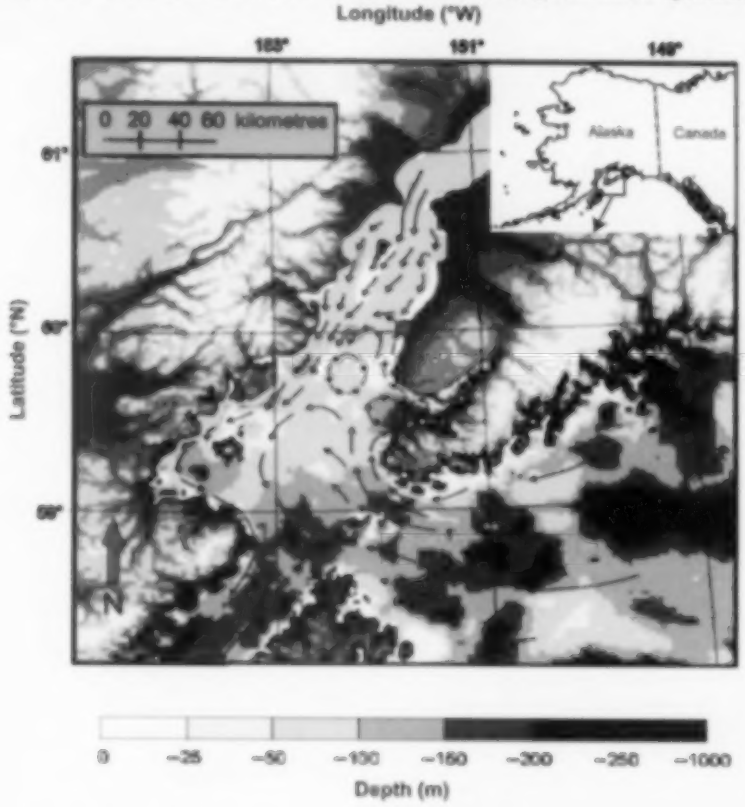
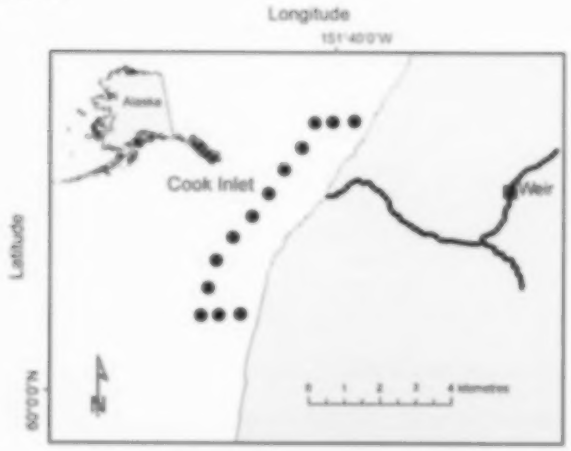


Fig. 2. Ninilchik River, Alaska, showing sampling weir and approximate locations for acoustic receiver moorings in Cook Inlet (circles).



checks were clearly defined and the scales not affected by regeneration or major resorption along the lateral axis. Mean Fulton's condition factor (K) was calculated for each life history type across all years (Guy and Brown 2007).

Acoustic tags

The freshwater-to-marine migration of postspawn steelhead kelts was studied using acoustic tags with individual fish identification codes and an array of moored acoustic receivers set in and around the mouth of the Ninilchik River in Cook Inlet. Ultrasonic transmitters (Vemco, Ltd., Nova Scotia; 9 mm × 30 mm, 5 g) were surgically implanted in the peritoneal cavity of 49 (2002) and 50 (2003) steelhead kelts. The acoustic tags were powered by a lithium battery and had a life expectancy of 125 days. Tags were programmed to transmit acoustic signals with a random pulse interval between 30 and 90 s. All tags transmitted at the same frequency (69 kHz), but each tag produced a unique six-ping coded signal so individual fish could be identified.

Surgeries were performed on a raised platform in the Ninilchik River just downstream of the weir. Appropriate measures were taken to minimize infection associated with surgery (Mulcahy 2003). A topical antiseptic, chlorhexidine diacetate, was used at the incision point followed by a 2.0 cm incision made anterior to the tip of the pelvic girdle on the midventral axis. The acoustic tag was inserted, gently massaged into position in the peritoneal cavity, and the incision was closed with four simple interrupted sutures. All tagged fish were held in a live box until they were alert and swimming freely (typically 5–24 min) and then allowed to volitionally leave the live box in the downstream direction.

Table 1. Demographic data on Ninilchik River steelhead kelts passing through the weir, 2002–2005.

	Kelts passed											
		Total fish			Tagged fish			Tag recoveries				
Year	Dates	*N*	Male (%)	Female (%)	*N*	Male (%)	Female (%)	Previously tagged (*N*)	PIT	Acoustic	Archival	Expelled
2002	24 May – 26 June	449	120 (27)	329 (73)	82	28 (34)	54 (66)	0	NA	NA	NA	NA
2003	16 May – 29 June	412	145 (35)	268 (65)	80	37 (46)	43 (54)	0	NA	NA	NA	NA
2004	13 May – 30 June	416	172 (41)	244 (59)	0	0	0	16	3/82	5/49	3/33	6/72
2005	6 May – 30 June	681	128 (19)	553 (81)	0	0	0	14	3/80	5/50	0/30	7/72

Table 2. General statistics on steelhead life history categories determined by scales.

											No. of relatedness loglikelihoods*		
LH type	Months at sea	*N*	Sex (M/F)	Mean FL (mm)	SD	Mean mass (g)	SD	Mean Fulton's *K*	SD	Gene diversity	Allelic richness	PO	FS	HS
FW males	0	7	7/0	479.4	45.8	118.4	30.4	0.104	0.002	0.531	2.91	0	0	0
First spawn	4–6	126	50/76	643.8	65.9	222.8	64.5	0.089	0.063	0.533	3.08	2	1	11
Consecutive	8–12	20	7/13	734.0	55.3	319.0	86.8	0.079	0.006	0.522	2.94	2	0	4
Alternate	16–20	14	5/9	714.5	57.8	289.3	76.2	0.077	0.006	0.547	3.13	2	0	2

Note: LH, life history; FW, fresh water; FL, fork length; SD, standard deviation; *K*, Fulton's condition factor.
*PO, parent offspring; FS, full sibling; HS, half sibling.

Acoustic tags were detected by ultrasonic receivers (Vemco, Ltd., VR2 single channel) that were deployed in a gate-like configuration surrounding the mouth of the Ninilchik River (see Fig. 2). The VR2 data loggers were suspended 3–5 m from the bottom of Cook Inlet at distances 0.65–1.88 km from the river mouth, approximately 0.5–2.8 km from shore. Receivers were deployed on short moorings with subsurface floatation and a surface buoy. Disposable, degradable anchors were used to secure the moorings to the bottom. Receiver arrays were deployed prior to tagging and recovered during the last week in June. In 2003, an additional receiver was deployed within the mouth of the Ninilchik River to gain accuracy on river migration times.

Each receiver contained 2 MB of flash memory and can operate up to 15 months on a single D-cell battery. Detection range of 0.5–1.0 km were expected using these receivers, depending on the power output of each tag. Mean distance between neighboring receivers was 710 m (range 283–806 m). The spacing of the receivers was based on global positioning system (GPS) coordinates that ensured overlapping detection ranges in an effort to minimize potential loss of detections between receivers. The first detection recorded by any of the receivers was classified as the time the fish entered salt water according to acoustic data. If fish were detected more than once, the total elapsed time was classified as the duration of residence near the mouth of the river. To determine if saltwater entry was random with respect to time of day, we used Rao's spacing test (Fisher 1993).

Archival tags

Archival tags containing temperature and pressure sensors (Lotek Wireless, Inc., LTD_1110; $N = 26$) and tags with temperature, pressure, and light sensors (Lotek Wireless, Inc., LTD_2410; $N = 37$) were used in this study. Archival tags were surgically implemented 4–20 June 2002 ($N = 33$) and 28 May – 18 June 2003 ($N = 30$) under the same surgical conditions as acoustic tags. Archival tags were cylindrical (3 mm long × 11 mm diameter; 5 g). A 5 cm plastic stalk was attached to the beta-type LTD_1110 tags to mimic active light stalks developed for the LTD_2410 tags by Lotek Wireless, Inc. A second small (2 mm) incision was made anteriorly adjacent to the tag insertion point to pass this stalk through the skin. The tag and stalk were secured by a single suture to the interior wall of the peritoneal cavity before closing such that the light stalk hung laterally along one side of the lower abdomen and extended to just above the vent of the fish. LTD_2410 tags used in 2003 were virtually identical in size and mass to beta-type LTD_1110 tags, but were equipped with a 10 cm functioning light stalk and equivalent sensor. Batteries used in the LTD_2410 were smaller. Battery technology also improved in the LTD_2410, and these tags were predicted to have up to a 3-year battery life. Electronic data from all sensors was stored in 32 kB of nonvolatile EEPROM, and all data records were correlated with an onboard clock.

Both types of tags were programmed to start recording at 15 s sampling intervals using a time extension recording program. When allocated memory was full for any sensor, every other sample in memory was overwritten with new time and date associated data. This memory extension program predicted that each tag would have approximately 32 760 data point storage capacity. Pressure data was programmed to have a maximum range of 3000 m with ±3 m accuracy. Standard depth calculations were corrected for gravitational density (latitude) and liquid density (temperature) using a formula from Lotek Wireless Inc., based on Harris (2000). The largest difference in estimated and corrected depth was 39 mm (0.13%); average depth correction was 3.8 mm. Temperature sensors were set to range between –5 and 35 °C with an accuracy of ±0.3 °C. Light sensors were programmed to record ambient light levels to

predict apparent sunrise and sunset and allow estimates of geolocation (approximate longitude and latitude) for fish during ocean migrations (Hill 1994; Welch and Eveson 1999). Unfortunately, the light stalk failed in the one archival tag with an active sensor recovered in this study, and no light data were analyzed.

Genetic analyses

Nonlethal caudal fin clips were randomly collected from outmigrating adults at the Ninilchik River weir for population genetic analysis in 2002 (N = 91), 2003 (N = 79), and 2005 (N = 126). Fin clips were stored in 100% ethanol and analyzed at the US Geological Survey Alaska Science Center's Molecular Ecology Laboratory, Anchorage, Alaska. Genetic samples were not collected in 2004 because of funding constraints. DNA was successfully extracted from 296 samples using the Puregene DNA Isolation kit (Gentra Systems, Inc.).

Eleven microsatellite loci were used to analyze genetic structure for Ninilchik River steelhead (Table 3). Loci were selected based on documented variability in *O. mykiss*, ease of polymerase chain reaction (PCR) amplification, and a history of allele scoring rigor (Table 3). Several primers were redesigned for use in this study: *Ogo4* forward (F), 5′-CAGAATCAGTAACGAACGC-3′; *Ogo4* reverse (R), 5′-GAGGATAGAAGAGTTTGGC-3′; and *Ots3* (R), 5′-CACAATGGAAGACCAT-3′). *Ogo1a*, *Ogo4*, and *Ots3* forward primers were modified by the addition of M13 (R) tails, and *Oneμ8* and *Oneμ11* forward primers were modified with M13 (F) tails. All M13 tails were added to the 5′ ends and allowed for allele fragment visualization by annealing to labeled complementary M13 tails added to the PCR mix. The remaining loci were visualized by adding directly labeled forward primers.

PCR reactions were conducted in 10–12 μL volumes using approximately 50 ng of genomic DNA, 0.06–0.1 U (1 U = 16.67 nkat) of *Taq* DNA polymerase (Promega), 10 mmol·L^{-1} Tris–HCl (pH 8.3), 1.5 mmol·L^{-1} $MgCl_2$, 50 mmol·L^{-1} KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100, 200 μmol·L^{-1} each dNTP, 0.1–0.5 pmol unlabeled primers, 0.1–0.4 pmol directly labeled primers, and 0.5–1.5 pmol labeled M13 tails. PCR reactions were carried out in MJ Research (BIORAD) or MWG thermocyclers (MWG Biotech Inc.), with an initial denaturation time of 2 min at 94 °C followed by 40 cycles of 94 °C for 15 s, 52 °C for 15 s, 72 °C for 30 s, and a final 30 min elongation step at 72 °C. The 30 min elongation step was not done for *Omy325*, *Oneμ14*, *Ots1*, and *Ots4*. Gel electrophoresis and visualization of alleles was performed using a LI-COR model IR2 automated fluorescent DNA sequencer, and allele sizes were assigned using GeneImagIR v. 3.00 software (LI-COR). Microsatellite allele sizes were quantified in relation to the M13 single nucleotide ladder, *O. mykiss* DNA samples of known size, and (or) the GeneScan-350 internal size standard (PE Biosystems). Approximately 10% of all samples were run on a second gel and scored independently for quality control.

Microsatellite Toolkit (Park 2001) was used to find matching samples among sampling years and create input files for subsequent analyses. GENEPOP v. 3.4 (Raymond and Rousset 1997) was used to test each locus for significant departures from Hardy–Weinberg equilibrium (HWE) and to provide a global estimate of HWE for all loci and all collection years combined. The significance level for interpreting HWE was adjusted for the number of loci using a Bonferroni correction (α = 0.0045; Rice 1989). ARLEQUIN v. 3.0 (Excoffier et al. 2005) was used to calculate observed (H_O) and expected (H_E) heterozygosity by locus and to calculate pairwise F_{ST} among three sampling years (θ, Weir and Cockerham 1984). Significance for pairwise F_{ST} was evaluated after applying a Bonferroni correction (α = 0.017). FSTAT v. 2.9.3 (Goudet 2001) was used to test for linkage disequilibrium among loci, and a Bonferroni correction based on the total number of comparisons (55) was used to evaluate significance (α = 0.0009). LDNE was used to estimate effective population size, N_e, for steelhead kelts in the Ninilchik River using all 11 microsatellite loci (Waples and Do 2008).

Genetic diversity within and among different life history types and between genders was analyzed using SAPGeDI (Hardy and Vekemans 2002). The program ML-Relate was used to calculate relatedness and predict relationship categories between all pairs of individuals using maximum likelihood (Kalinowski et al. 2006). We assessed 39 060 relationships among all unique individuals in our genetic database. Overall relatedness among Ninilchik River steelhead was determined by averaging r values for all comparisons.

Results

Demographic data

The number of steelhead kelts that passed through the weir each year varied significantly (average = 490 fish; Table 1). Outmigrating female kelts outnumbered (≥59%) male kelts each year. Male–female ratios ranged from 1:1.4 (2004) to 1:4.3 (2005). Male–female ratios in tagged fish were 1:1.9 (2002) and 1:1.2 (2003). Six PIT tags (3.7%), ten acoustic tags (10.1%), and three archival tags (4.8%) were recovered from adult steelhead in subsequent years at the weir (Table 1). Thirteen downstream migrants showed various signs of previous surgeries (remaining sutures or surgical scars) with no indication of remaining tags. We assumed all tags from these fish were expelled during sea migrations or at spawning. All recovered tagged fish were found at the weir 2–3 years after the year they were tagged.

Four different migratory life histories were observed in adult steelhead scales based on growth patterns. Scales taken from two kelts (~1%) showed regeneration and were excluded from life history analyses. Migratory life histories included (*i*) freshwater adults with no seawater history and no evidence of prior spawning or residual gametes; (*ii*) kelts that were returning to sea after their first spawning event; (*iii*) repeat spawning steelhead that reproduced in consecutive years (after one summer and fall at sea between spawning events ~8 months at sea); and (*iv*) repeat spawning steelhead that reproduced in alternate years (~16 months at sea). All seven freshwater adults captured at the weir were male, and these fish appeared to be headed to sea for the first time, although actual sea entry was not confirmed because these fish were not tagged. We have no empirical data to confirm reproduction in fresh water by these males prior to migration. First time spawning steelhead dominated

Table 3. Descriptive statistics and sources for microsatellite loci used to evaluate Ninilchik River steelhead.

Locus	Source	N_A	Allelic size range	H_O	H_E	P for HWE
Ogo1a	Olsen et al. 1998	4	132–160	0.646	0.649	0.532
Ogo4	Olsen et al. 1998	4	118–134	0.596	0.623	0.678
Omy27	Heath et al. 2001	4	103–117	0.513	0.531	0.760
Omy77	Morris et al. 1996	7	103–133	0.657	0.678	0.019*
Omy325	O'Connell et al. 1997	7	95–149	0.675	0.671	0.629
Oneμ8	Scribner et al. 1996	3	152–172	0.475	0.459	0.585
Oneμ11	Scribner et al. 1996	3	145–149	0.262	0.259	0.782
Oneμ14	Scribner et al. 1996	5	147–155	0.573	0.580	0.086
Ots1	Banks et al. 1999	7	159–243	0.531	0.536	0.142
Ots3	Banks et al. 1999	4	81–89	0.618	0.633	0.870
Ots4	Banks et al. 1999	6	108–130	0.279	0.292	0.329

Note: Data includes the number of alleles (N_A), allelic size range in base pairs, observed (H_O) and expected (H_E) heterozygosity, and P values for interpreting Hardy–Weinberg equilibrium (HWE).
*$P \geq 0.0045$ indicate the locus is in HWE after Bonferroni correction.

the postspawn downstream steelhead migration (58%). Based on tag recoveries (all types), clearly identifiable suture or insertion scars, and (or) PIT tag retention, we were able to document that 17% of the tagged steelhead from 2002 were recovered in 2004 and 20% of the tagged steelhead in 2003 were recovered in 2005. Consecutive-year steelhead outnumbered alternate-year steelhead at the weir in both years. Females that spawned more than once were found in all years and across all life history types in the surviving fraction of repeat spawners. Males were found in each repeat spawning category, but not in all years. Fish of both genders may have been lost to predation or natural death during or after spawning.

Mean condition factor (K) was higher in freshwater males without sea experience, but mean K was not statistically different among the four life history types (analysis of molecular variance, AMOVA F value < 0.0001; $P > F = 0.938$). The four highest K values were found in first spawn female kelts ($K = 0.11$–0.14). In general, females did not have significantly higher condition factors than males in years when lengths and masses of outmigrating steelhead kelts were monitored during tagging (2002–2003), but female condition did have greater variance in both years: mean $K \pm$ standard deviation (SD) for females in 2002: $K = 0.079 \pm 0.012$; males in 2002: $K = 0.087 \pm 0.009$; t test assuming unequal variance ($t = 0.01$; $P = 0.44$). Mean K values for females in 2003 was 0.096 ± 0.207; mean K values for males in 2003 was 0.087 ± 0.008 ($t = 0.28$; $P = 0.16$). There were no significant trends by gender for temporal downstream kelt migrations for fish measured at the weir during 2002–2003: 25 May – 5 June (early) males $N = 30$, females $N = 44$; 6–30 June (late) males = 44, females = 50. No significant differences in K were found for early or late migrating kelts (both genders combined; t test assuming unequal variance; 2002: $t = 0.27$, $P = 0.19$; 2003: $t = 0.36$, $P = 0.32$).

Acoustic tags

Kelts tagged in 2002 ranged in length from 450 to 782 mm, and kelts tagged in 2003 ranged in length from 498 to 870 mm. The first fish was tagged on 30 May (2002) and on 25 May (2003). Detections of individual fish by the buoy array occurred until 21 June (2002) and 23 June (2003). Not all fish tagged with acoustic transmitters were detected by the automated receivers. A total of 38 (76%; 2002) and 46 (92%; 2003) tagged fish were detected leaving the Ninilchik River. In 2003, 48 (96%) fish were detected by the receiver located in the lower river before saltwater entry. Two acoustic-tagged fish were recorded in the lower river, but not in the coastal marine array. Eighty-six percent of acoustic tags were recorded by receivers for 2002 and 2003 combined. Ten acoustic tags were recovered from kelts returning to the weir in 2004 and 2005 (five tags each year).

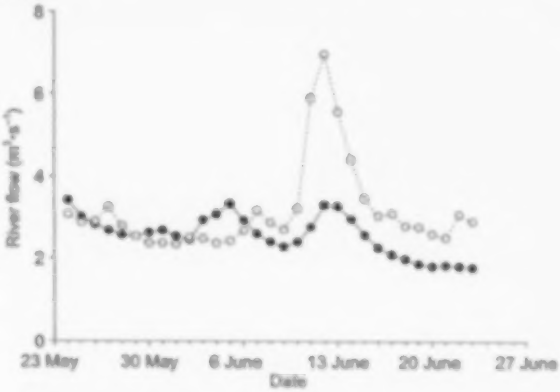
Daily mean flow during the period that acoustic-tagged steelhead kelts migrated out of the Ninilchik River ranged from 1.78 to 3.43 $m^3 \cdot s^{-1}$ in 2002 and from 2.35 to 6.97 $m^3 \cdot s^{-1}$ in 2003 (Fig. 3). Transit time between the weir and saltwater (i.e., time that elapsed between after-tagging release and first detection in salt water) ranged from 8.6 to 254.9 h. There was no relationship between flow on the day of tagging and transit time (2002: $r^2 = 0.03$, $n = 38$, $P = 0.29$; 2003: $r^2 = 0.12$, $n = 46$, $P = 0.02$). Mean rate of travel (±SD) between the tagging site and the receiver array was 5.10 ± 4.40 km·day^{-1} and 8.55 ± 9.94 km·day^{-1} in 2002 and 2003, respectively. There was no relationship between the length of fish and transit time (years and sexes combined: $r^2 = 0.10$, $n = 84$, $P = 0.01$). The mean rate of downstream travel (±SD) of female fish in 2002 was 3.98 ± 1.69 km·day^{-1}, and the mean rate of downstream travel of male fish in 2002 was 7.24 ± 6.83 km·day^{-1}. In 2002, male kelts migrated significantly faster than females ($t = -2.28$, df = 36, $P = 0.01$). In 2003, mean rate of travel was 9.33 ± 9.66 km·day^{-1} for female kelts and 7.44 ± 10.48 km·day^{-1} for male kelts. In 2003, there was no significant difference in transit time between male and female kelts ($t = 0.6228$, df = 44, $P = 0.54$).

Steelhead kelts remained within the vicinity of the receiver array from 0 to 35.5 h. Time spent near the shore as determined by acoustic tags (±SD) averaged 1.47 ± 1.1 h in 2002 and 1.95 ± 0.9 h in 2003. There were no significant differences in duration that fish remained near the mouth of the river when comparing between years or between sexes. Similarly, there was no relationship between fish length and

the length of time fish were held near the mouth of the river. In both years, fish movement from fresh water to salt water occurred primarily between the hours of midnight and 18:00, with only one acoustic-tagged fish in 2002 and two acoustic-tagged fish in 2003 making the transition between 18:00 and midnight (Fig. 4). Mean time of saltwater entry was 07:29 in 2002 and 06:01 in 2003. Timing of saltwater entry was not uniformly distributed throughout the day (Rao's spacing test 2002: $U = 148.8$, $n = 38$, $P < 0.05$; 2003: $U = 163.3$, $n = 38$, $P < 0.01$).

Migration of kelts from fresh water to salt water was related to tidal stage with 68% of fish making the transition from fresh water to salt water at high tide and on the ebbing flow (Fig. 5). When the tide cycle was divided into three 4 h periods (i.e., the flood, high, and ebb tides), significantly fewer entries into saltwater occurred during the hours of incoming flood tide in both years (χ^2 test 2002: $n = 36$, $P > 0.0001$; 2003: $n = 46$, $P = 0.02$).

Fig. 3. Discharge ($m^3 \cdot s^{-1}$) in 2002 and 2003 during the period of migration by acoustic-tagged steelhead kelts, Ninilchik River, Alaska. Solid circles are data from 2002; open circles are from 2003.



Archival tags

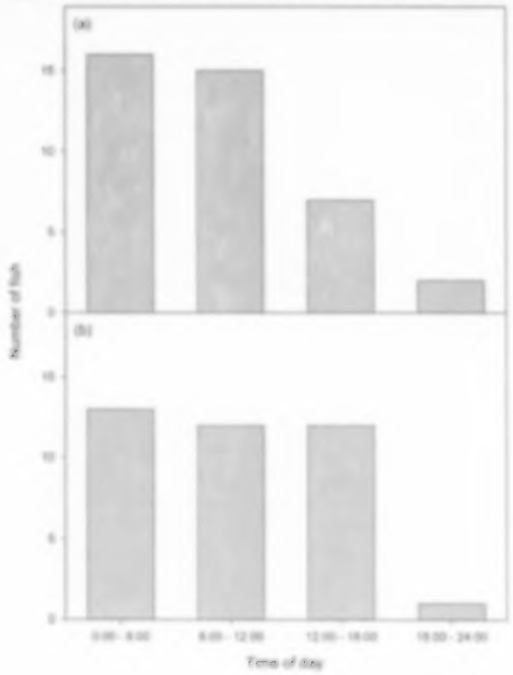
Tag mass to fish mass ratios fell below 1% in all surgeries. Two archival tags (LTD_1110) implanted in 2002 and recovered in 2004 from alternate-year kelts provided data on temperature and depth at 30 min intervals for steelhead at sea for 16 months and during their upstream marine–freshwater migrations. One LTD_2410 collected from a kelt in 2004 was corrupted and no data were downloaded from the sensors. We were unable to determine what caused this malfunction, but the tag was probably corrupted by seawater through the light stalk attachment point to the tag body where the light stalk was bent at the time of recovery. One steelhead captured at the weir in 2005 had a recognized PIT tag, indicating that this fish had been tagged with a LTD-2410 archival tag in 2002, but no archival tag was found in the peritoneal cavity. We assume this archival tag was expelled. Estimated minimum expulsion rate in repeat spawning kelts based on sutures, scars, and remaining PIT tags observed in archival-tagged kelts was 22% (11 females and 3 males). But this is clearly an underestimation, since several previously tagged fish lacked associated PIT tags or suture pattern to clearly indentify the type of original tag placed in the peritoneal cavity, and others may have healed without identifiable scars.

The two archival tags recovered from steelhead kelts recorded sensor data for 711 (male) to 716 (female) days at sea (Table 4). Condition factor (K) between the time of tagging and at recovery 2 years later (postrepeat spawning) increased in the female kelt ($K = 0.077$ to 0.102) and decreased in the male kelt ($K = 0.084$ to 0.077). Archival tags demonstrated that these kelts spent approximately 97% of their time at sea in marine waters less than 6 m deep (day and night), with the greatest proportion of time spent between 3 and 4 m depth (Table 5).

While at sea, 94% of time spent at moderate depths (6–20 m) took place during summer daylight hours (10:00–20:00) in both fish. The male kelt's archival tag (No. 1563) recorded 134 short-duration dives over 20 m, while the female kelt's tag (No. 1560) only recorded 27 dives below 20 m depth. The male kelt also spent significantly more time at depths ≥ 20 m (total = 94 h; average cumulative time at each 2 m depth interval = 11.75 h) than the female kelt (total = 17.5 h; average cumulative time at each 2 m depth interval = 2.19 h; Table 5). The male kelt's tag recorded the three deepest dives (88.7 m (02:41 on 27 November 2002), 73.9 m (10:11 on 5 February 2003), and 50.5 m (21:41 on 25 September 2003). The deepest depth recorded for the female fish (32.08 m) occurred on 25 September 2002 at 03:43.

Both tags revealed seasonal activity at depth with frequent dives from July to early September during both summers at sea and little activity at depth during spring and winter (Table 6; Fig. 6). Our records of kelts at depths ≥20 m were most common during the month of August for both fish in both summers at sea when temperatures were higher, especially during dusk and night. However, the timing and regularity of deep dives varied, suggesting a lack of consistent crepuscular diving behavior. Variation in the average temperatures recorded during surface swimming (0.3–4.0 m) during their first 2 months at sea for the female (8.8 °C) and male (10.4 °C) steelhead suggest that they may have spent that time in different parts of the ocean or at different depths during this time. Temperature–depth profiles also varied during the steelhead's time at sea, suggesting unique patterns of activity, but no general seasonal or temporal pattern was observed in these profiles (Fig. 7). Minimum winter temperatures at sea recorded in December 2002 (6.23–6.94 °C) suggest a possible maximum southern distribution between latitudes 44°N and 45°N in the North Pacific Ocean based on sea surface temperature (SST) reference data reported for that year (Fig. 8), assuming these fish stayed in the northeastern Pacific Ocean. Fall 2002 SST reference values reported for marine waters geographically proximate to the Ninilchik River and in lower Cook Inlet (Okkonen and Howell 2003) also parallel temperatures recorded by archival tags during that time, suggesting these fish may not have migrated out of Cook Inlet during this marine stage. Hedger et al. (2009) also reported long-term residence of many Atlantic salmon kelts in nearshore delta waters, suggesting the need to improve somatic reserves prior to seaward migrations. Steep diurnal temperature fluctuations recorded by the archival tags temperature sensors in fresh water and reduced variation in diel temperatures experienced

Fig. 4. Number of steelhead kelts entering salt water in relation to time of day in (*a*) 2002 and (*b*) 2003, Ninilchik River, Alaska.



at sea clearly delineated the fish's migration to and from salt water (Fig. 9).

Genetics

Genetic tissues were taken from 18%–20% of all steelhead kelts passing through the Ninilchik River weir in each of 3 years (Table 7). Thirteen genetic samples collected in 2005 shared identical multilocus genotypes to other samples previously collected in our database. Identical samples were removed from statistical analyses of data pooled across sampling years, resulting in a total of 280 unique individuals in our genetic analyses. Ninilchik River steelhead kelts conformed to HWE for all loci and all samples combined (P = 0.33; χ^2 = 24.3). Average H_O across all loci was 0.53. The average number of alleles per locus was 4.9. No linkage disequilibrium was detected between loci ($P > 0.0009$ for all pairwise comparisons). Estimates of effective population size (N_e) were similar to actual weir counts in 2002 and 2005, but differed significantly in 2003 (Table 7). Pairwise F_{ST} comparisons ranged from –0.001 to 0.002, and no significant genetic differentiation was detected among sampled years.

Overall average relatedness among Ninilchik River kelts was r = 0.1. All possible paired relationships were assessed among the 280 kelts with unique genotypes. Of these, 5.8% (2265) were predicted to be parent–offspring pairs, 3.1% (1194) full-siblings, and 16.4% (6409) half-sibling pairs. The majority (74.7%) of all paired relationships were considered unrelated (29 192). We tested for significant differences in genotypes among life history types despite the fact that allelic diversity at these 11 loci was relatively low

Fig. 5. Number of steelhead kelts entering salt water in relation to tidal stage in (*a*) 2002 and (*b*) 2003, Ninilchik River, Alaska.

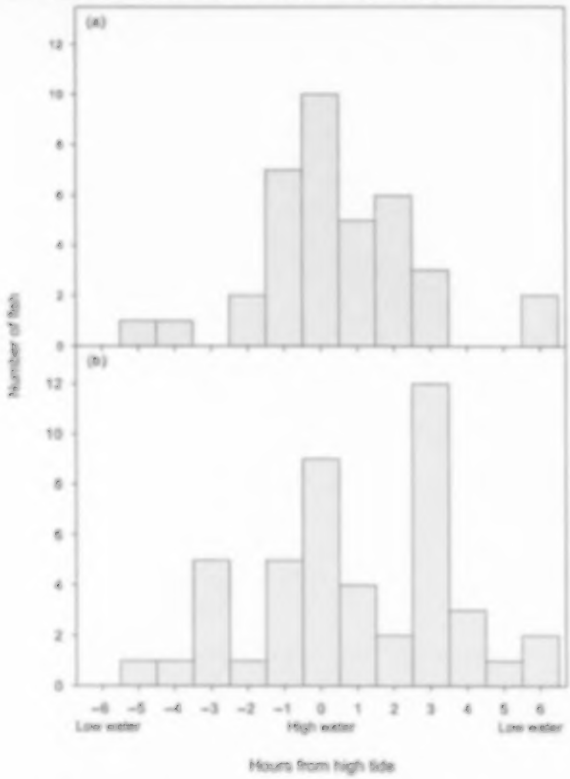


Table 4. Archival tag records for 16 months at sea for two Ninilchik River repeat spawning steelhead kelts, 2002–2004.

	Tag No.	
	1560	1563
Sex	Female	Male
Release date	9 June 2002	20 June 2002
Length (mm)	574	630
Mass (kg)	1.45	2.1
Recovery date	26 May 2004	1 June 2004
Length (mm)	672	755
Mass (kg)	3.1	3.3
Days at large	716	711
Minimum depth (m)	0.3	0.6
Maximum depth (m)	32.1	88.7
Minimum temperature (°C)	4.9	3.9
Maximum temperature (°C)	13	13

(average number of alleles = 4.9) and associated sample sizes were small, reducing the power of these analyses. Alternate-year repeat spawning adults (N = 14) were shown to be genotypically different from first time spawning adults (N = 126) across all years using AMOVA (F_{ST} = 0.015; P = 0.032), but this differentiation was not statistically significant when corrected for multiple tests using Bonferroni (significant α = 0.0125). No other patterns of genetic differentiation among life history types were found.

Table 5. Time at depth (cumulative hours) for steelhead carrying archival tags Nos. 1560 (female, F) and 1563 (male, M).

Depth interval (m)	Tag No. 1560 (F)	Tag No. 1563 (M)
2.00	768.00	612.00
4.00	9028.50	9573.50
6.00	658.50	370.50
8.00	113.00	57.50
10.00	101.50	51.00
12.00	113.00	53.50
14.00	89.00	65.00
16.00	25.50	77.00
18.00	18.00	36.50
20.00	5.00	29.00
22.00	7.50	20.50
24.00	2.50	21.50
26.00	1.50	11.50
28.00	0.50	3.00
28–29.99	0.00	2.00
30–31.99	0.00	3.00
>32	0.50	3.50

Note: Data is for marine migrations only.

Discussion

Information about downstream migration and survival of steelhead kelts is very limited (see however Narum et al. 2008). In recent years, more effort has been extended to increase survival of steelhead kelts passing through managed rivers systems such as the Columbia River with a desire to increase overall population size, presumably with increased reproductive success through repeat spawning especially in poor recruitment years or in recently bottlenecked populations (Ward and Slaney 1990; Evans et al. 2004; Keefer et al. 2008). These studies suggest that further work is needed to address the physiology and behavior of steelhead kelts as they migrate between fresh water and salt water. Acoustic tags and automated receivers were successful in this study in describing several factors that provide important information on natural kelt migration to the sea from the Ninilchik River where there are no dams or physical passage problems. Most kelts moved rapidly to salt water after passing the weir with no evidence of difficulty in adaptation to marine conditions despite the energy demands of repeat spawning and their prolonged period in fresh water (up to 10 months). No relationship was found between the length of fish and transit time for Ninilchik River kelts, unlike findings for brown trout (*Salmo trutta*) kelts by Bendall et al. (2005). In 2002, male kelts migrated substantially earlier than females, similar to results reported for Atlantic salmon kelts in Norway by Halttunen et al. (2009). This was not the case for male kelts in 2003. Females did not appear to migrate earlier and condition was not associated with migration.

Bendall et al. (2005) found that migration of brown trout kelts through an estuary was predominantly nocturnal, with most fish migrating through the estuary after midnight. Migrations of brown trout in darkness were assumed to greatly reduce the risk of visual predators. Movement of steelhead kelts from fresh water to salt water on the Ninilchik River was diurnal, with few fish moving between the hours of 20:00 and midnight. This pattern in steelhead kelts may be related to water temperature. Maximum daily water temperatures, as measured by an automated data logger in the river just upstream of the mouth, ranged from 9.2 to 16.2 °C during the study period, and daily high temperature typically occurred between the hours of 17:00 and 20:00. Steelhead have been shown to behaviorally adjust activity rates and metabolic demand in response to high water temperatures (Coutant 1985; Nielsen et al. 1994). Decreased detections, therefore, may reflect decreased activity in response to periods of higher water temperatures. Comparisons of our observations to the limited literature available on kelt migrations seem to indicate that trends and condition for kelts during their downstream migration may not be uniform across species and locations or even across years at the same location.

Kelt seaward movement was positively associated with tidal phase as previously reported in other studies (Bendall et al. 2005; Hedger et al. 2009). Two fish recorded in the lower river in 2003, but not in the coastal marine array, may indicate that some fish escaped detection by our array. This could also have resulted from illegal harvest of kelts at the river mouth. Acoustic tag reporting rates during the 2 years of deployment (86%) were lower than rates for Atlantic salmon kelts (95%; Halttunen et al. 2009). Survival rates are difficult to estimate because of possible detection errors. Several unpredictable factors may have confounded our 2002 acoustic tag data. Of the 12 tags that were not detected in 2002, nine were tagged 1 or 2 days prior to weekends during a sport fishery for Chinook salmon on the lower Ninilchik River, late May to mid-June. Steelhead were authorized as catch-and-release only on this river, but species identification by some anglers was questionable, and it is possible that steelhead were harvested (N. Szarzi, Alaska Department of Fish and Game, 3298 Douglas Place, Homer, AK 99603, USA, personal communication, 2005). We suspected that some of the tagged fish were captured in this illegal fishery. Therefore, in 2003, we avoided tagging fish prior to weekends during the Chinook fishery. This may explain differences in detection rates between the 2 years (2002 = 76%; 2003 = 96%).

The lack of recovered archival tags in repeat spawning kelts greatly compromised this portion of our study. High rates of expulsion of surgically implanted acoustic and telemetry tags have been documented in other studies (Jepsen et al. 2002; Lacroix et al. 2004). Tags have been shown to become encapsulated in a thick membrane and then expelled through the body at the surgical incision, at the abdominal wall adjacent to the healed incision or where the pressure of the tag was greatest (Lucas 1989). Tags can also be expelled through transintestinal expulsion and during spawning by passage out the oviduct (Moore et al. 1990; Jepsen et al. 2008). Suture scars and healed incision scars were obvious in some previously tagged kelts passing though the weir after their second spawning cycle. No other obvious body scars related to expulsion were evident. We suspect that most tags in steelhead kelts were expelled during spawning, but we did not find any expelled tags during several surveys of upstream spawning habitat using metal detectors. We could easily have missed tags expelled during spawning, or they could have washed downstream into areas not surveyed.

Table 6. Monthly mean, standard deviation (SD), maximum and minimum temperatures (°C), and depths (m) of steelhead archival tags Nos. 1560 (female, F) and 1563 (male, M), June 2002 through September 2003.

		June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Total
Temperature																		
1560 (F)	Mean	8.7	9.2	9.8	8.7	8.1	7.1	7.0	7.0	5.8	6.1	6.3	6.0	7.5	10.0	11.7	11.4	8.0
	SD	0.9	0.5	0.5	0.8	0.7	0.2	0.4	0.7	0.3	0.6	0.6	0.5	1.3	0.5	0.5	0.7	1.9
	Maximum	10.9	10.7	10.9	10.3	9.2	7.6	7.9	8.1	6.4	7.6	7.9	8.1	10.9	11.2	12.9	13.0	13.0
	Minimum	6.4	7.9	8.3	6.8	7.0	6.6	6.2	5.5	5.3	5.3	4.9	5.3	5.7	8.9	10.5	9.8	4.9
1563 (M)	Mean	9.4	10.7	10.1	9.1	8.1	6.6	6.2	6.2	5.5	5.0	6.1	5.8	6.5	9.2	11.8	11.5	7.9
	SD	0.8	0.7	0.4	0.4	0.4	0.6	0.5	0.7	0.7	0.6	0.3	0.4	0.5	0.8	0.5	0.9	2.3
	Maximum	12.1	13.0	11.3	9.9	8.9	7.6	7.6	7.8	7.2	6.3	7.4	7.2	7.6	10.6	12.8	13.0	13.0
	Minimum	8.4	7.8	7.4	7.4	7.4	5.7	5.5	4.9	4.1	3.9	5.5	5.1	5.3	7.4	9.9	8.4	3.9
Depth																		
1560 (F)	Mean	1.0	2.4	4.1	3.2	3.4	3.6	3.6	3.6	3.9	3.8	3.9	3.9	3.8	5.4	5.2	4.4	3.8
	SD	0.7	1.9	3.7	0.9	0.4	0.9	0.6	0.3	0.5	0.6	0.3	0.8	0.6	2.9	2.6	1.7	1.8
	Maximum	10.9	24.2	26.4	32.1	6.9	20.8	12.4	6.9	10.9	17.1	8.0	21.8	13.5	16.8	18.6	17.9	32.1
	Minimum	0.3	1.0	2.1	2.9	2.5	2.9	3.2	3.2	3.2	3.6	3.6	3.6	3.6	3.6	3.5	3.5	0.3
1563 (M)	Mean	1.0	2.6	4.6	3.2	3.5	3.5	2.8	2.3	2.5	3.2	3.7	3.2	3.1	4.5	5.6	3.8	3.5
	SD	0.6	2.1	4.0	0.7	1.1	2.4	0.7	0.5	2.0	0.8	0.7	0.9	1.4	3.6	5.9	3.2	2.7
	Maximum	5.7	20.2	30.5	14.4	21.2	88.7	8.4	5.3	73.9	5.4	17.8	13.1	19.4	25.5	33.2	50.5	88.7
	Minimum	0.6	0.9	2.2	2.4	2.7	2.8	2.0	2.0	2.1	2.1	2.1	2.1	2.0	1.8	1.6	1.6	0.6

Note: Statistics exclude data from fresh water, October 2003 through June 2004.

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Fig. 6. Dive patterns and time at depth recorded by two archival tags in steelhead kelts while at sea during the summer of 2003. Panel (*a*) depicts data collected from tag No. 1560, and (*b*) shows data collected from tag No. 1563.

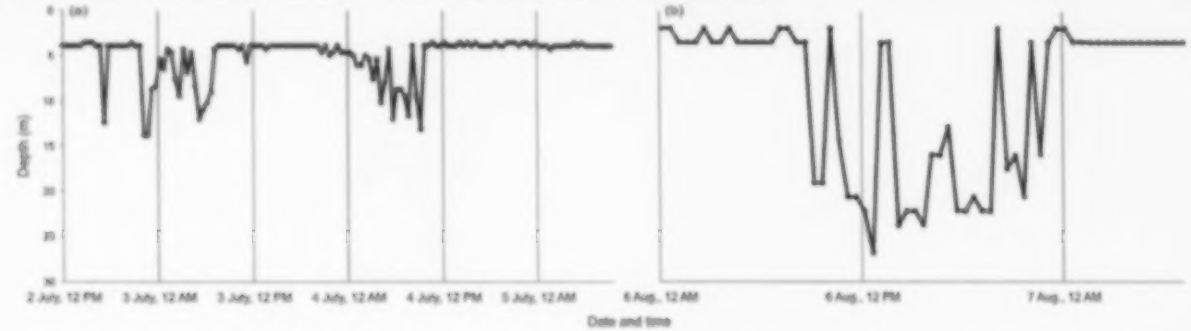
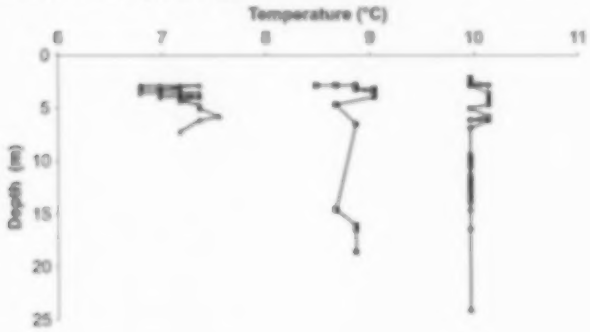


Fig. 7. Temperature–depth profiles for three different vertical migrations performed by a steelhead kelt (tag No. 1560) on three days during time at sea. Circles represent data collected 31 July 2002, squares represent data from 29 August 2002, and triangles represent data from 25 September 2002.



In several laboratory studies, only limited near-term mortalities or obvious infection occurred as a result of tag expulsion (Moore et al. 1990; Lacroix et al. 2004; Welch et al. 2007), but it is difficult to judge tag expulsion rates and effects in the wild. Tag mass relative to fish mass has been shown to be an important factor determining tag retention in many studies (Jepsen et al. 2005). We tagged large adult steelhead and our tag-to-fish mass ratio (<1%) was within even the strictest recommendations found in the literature (Summerfelt and Mosier 1984; Perry et al. 2001). We cannot estimate actual mortality for tagged kelts at sea. Therefore, it is not possible to differentiate tag loss due to natural ocean mortality as opposed to expulsion mortality, but based on observations of repeat spawning kelts with scars it seems clear that adult steelhead can physiologically process alien material implanted into the peritoneal cavity to pass tags. Additional research on how iteroparity can affect tag loss is needed.

Even with limited sample sizes, data obtained from two steelhead kelts carrying archival tags were very informative. Decreased movement of adult steelhead at night as they migrated from the ocean into fresh waters was reported by Ruggerone et al. (1990). Archival tags recovered from kelts showed that both fish migrated into fresh water in late September (2003) between 14:00 and 17:30. While ocean migration data are beginning to accumulate for some Pacific salmon (Friedland et al. 2001; Tanaka et al. 2005; Walker et al. 2007), there is very limited data on the movements and behavior of steelhead at sea (Walker et al. 2000; Davis et al. 2008).

Activity at depth was most common in the late summer at sea, with little movement to depth during the winter months. Seasonal ocean surface temperatures vary only slightly between Cook Inlet and the North Pacific Ocean. Minimum winter temperatures recorded by the archival tags gave us only inexact indications of possible broad geographic locations during winter. These fish spent a large proportion of their time at sea at the surface, and even the deepest records reported by the tags could be found in Cook Inlet, so depths may not be directly correlated to bathymetry in the North Pacific Ocean. Depth and temperature profiles demonstrated unexpected relationships that may be linked to variation in seasonal forage strategies. Numerous movements at depth recorded just after seawater entry and just before freshwater reentry seem to indicate levels of activity that could be associated with feeding behavior due to high bioenergetic demands before and after spawning or to orientation behavior upon entry and exit from different water bodies. Swimming behavior at depths >20 m occurred throughout the kelts' time at sea. Unlike diving patterns shown in many deep-sea pelagic fishes (Block et al. 2001), steelhead kelts demonstrated no consistent crepuscular pattern of deep dives during dawn and dusk periods. Hedger et al. (2009) reported diving behavior in Atlantic salmon was more frequent during daytime. Most steelhead kelt swimming behavior >20 m took place during daylight hours in August for each year at sea, although many of the deepest locations for steelhead (>32 m) were recorded at night, August – September 2003.

The most intriguing data drawn from these two tags was our finding that ocean-migrating steelhead kelts spent 97% of their time at sea in the top 6 m of the ocean, with the greatest time at depth between 3 and 4 m. Ruggerone et al. (1990) demonstrated that adult steelhead swam primarily at the surface (72% of time in the top 1 m) in a coastal fjord in British Columbia, regardless of salinity and temperature. Walker and Myers (2009) reported on a Yukon River Chinook salmon that spent its first summer at sea in the top 50 m. In other studies, Hubley et al. (2008) and Halttunen et al. (2009) demonstrated near-surface migrations of Atlantic salmon kelts in fjord and coastal habitats immediately following seawater entry, but no data were available for

Fig. 8. Contour plot of generalized average sea surface temperatures (SST) for December 1982–2002, observed in the Northeast Pacific Ocean based on data compiled by Fisheries and Oceans Canada (DFO), Oceans and Aquaculture Science Branch. Generalized average SST values ranged from 4 to 20 °C. Warmer ocean temperatures are shown in red. (Graphic adapted from DFO Ocean Sciences Web site: http://www-sci.pac.dfo-mpo.gc.ca/data/sst_data/clima/December.gif; accessed 6 August 2010.)

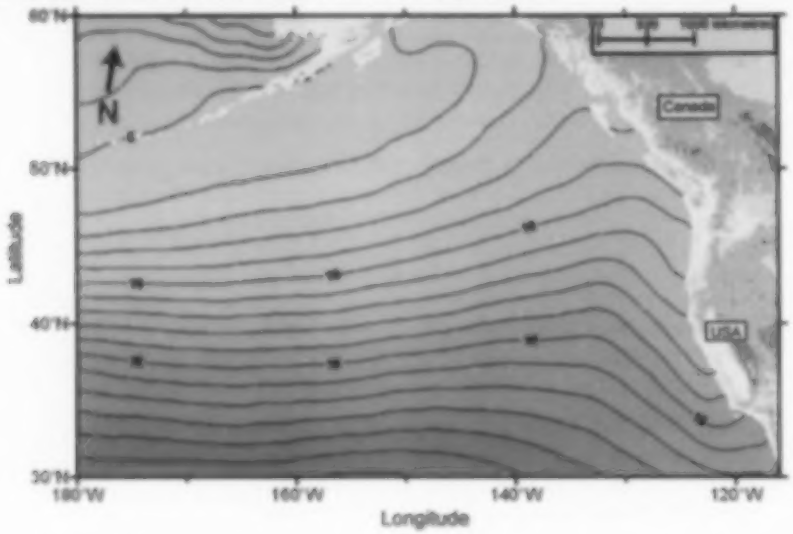


Fig. 9. Patterns of migration timing to the ocean and back to fresh water for repeat spawning steelhead kelt recorded by archival tag No. 1560. Arrows indicate movement from fresh to salt water determined by the dramatic drop in diel temperature variance when the fish enters salt water and the reverse migration some 15 months later. (*a*) June 2002, (*b*) September 2003.

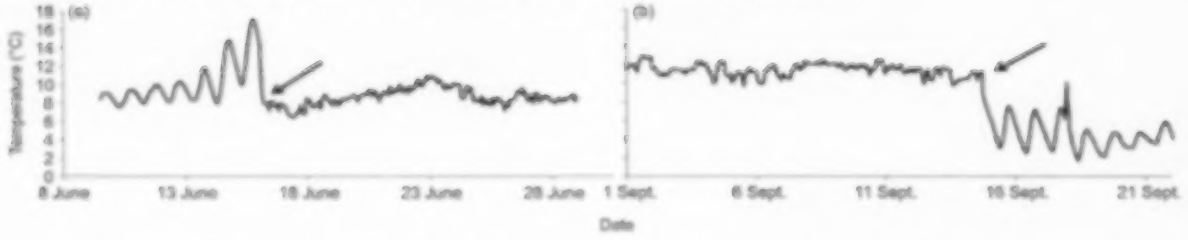


Table 7. Estimates of effective population size (N_e) and weir counts for Ninilchik River steelhead kelts in 2002, 2003, and 2005.

Collection year	Genotype (N)	Weir count	N_e	N_e:N (weir)
2002	91	449	398	0.89
2003	79	412	128	0.31
2005	123	681	565	0.82

Note: Values of N_e for the three sampling years reflect the removal of 13 identical genotypes found within the database in subsequent years. No genetic samples were collected in 2004.

open ocean migrations in these studies. These studies together with our data on near-surface swimming behavior for steelhead kelts during 16-month ocean migrations suggest unique marine migratory behavior in iteroparous kelts that differs from that reported for other salmonids (Walker et al. 2007).

Theoretically, there is a direct relationship between the degree or scale of repeat breeding in iteroparous species and reproductive investment at each breeding event (Crespi and Teo 2002). The female kelt in this study was more conservative in her behavior at depth and spent less time below 20 m depth when compared with the male. This depth is well above the typical thermocline for the Gulf of Alaska during the winter when the surface mixed layer is typically greater than 35 m (Stabeno et al. 2004). The relationship between iteroparity and reproductive investment at sea may be gender-specific in Alaskan steelhead. Actual biological and physical factors leading to differences in behavior at depth between these two fish would have to be confirmed with a larger sample size of tagged kelts from both sexes and associated geolocation data to test the alternative hypothesis that these fish were simply swimming in very different marine environments. Geolocation appears to be a critical missing element in the study of salmonid ocean behavior using archival tags.

The diversity of iteroparity shown by steelhead in this study was complex and highly variable. Other forms of iteroparity have been reported in the literature for salmonids (Crespi and Teo 2002; Hendry et al. 2002; Keefer et al. 2008). Genetic analyses, however, showed no significant differences in population structure or allelic richness among

any of the life history types found in repeat spawning kelts. Most kelts were not highly related based on familial relatedness likelihoods. Parent–offspring, full-sibling, and half-sibling relationships were few and evenly spread across the different life histories. All the genetic evidence suggested a heterogeneous population lacking unique structure based on spawning history or type of iteroparity. This result supports previous evidence that population genetic structure in *O. mykiss* is based on homing and evolutionary history rather than relative scales of anadromy, iteroparity, or life history type (Heath et al. 2001; Narum et al. 2004; Thrower et al. 2004).

Typical in-river spawning mortality rates are unknown in Alaskan steelhead, and the numbers of repeat spawning adults vary year to year on any given stream (Johnson and Jones 2001). The number of iteroparous steelhead found in this study was not substantially different from those reported in several other southeast Alaskan streams making up 20%–70% of the upstream migration (Lohr and Bryant 1999). Declines in an individual fish's ability to spawn at age are expected because of the cumulative energetic demands of migration and total energy costs of iteroparity (Berg et al. 1998). Differences in compensatory growth, reproductive development at sea, and body condition after spawning may explain why some iteroparous kelts returned to spawn again in consecutive or alternate years (Rideout et al. 2005; Hubley et al. 2008). Other physiological or epigenetic mechanisms may also contribute to this variation. Jonsson et al. (1991, 1997) indicated an association between body size (or energy content) and the tendency of Atlantic salmon to spawn annually or biennially; however, we found no differences in mean body mass for consecutive and alternate steelhead life history types.

Other studies have suggested that there is significant gender variation in the degree of iteroparity in steelhead, with more females spawning multiple times than males (Burgner et al. 1992; Jonsson and Jonsson 1993; Wertheimer and Evans 2005). We found no evidence that females participated in multiple-year spawning at a higher rate than males. Highly dynamic and productive ecosystems found in southeast Alaskan marine waters may provide sufficient resources to allow multiyear spawning for both genders in Alaskan steelhead. We cannot estimate individual survival or the relationship between size and spawning success without data on sex ratios, length, and masss of upstream migrants, which were not available in this study. Genetic estimates of effective population size (N_e) were similar to the counts of downstream migrating steelhead adults at the weir in 2 of the 3 years when genetic samples were taken. N_e results in 2003 were significantly lower than the weir count, suggesting a smaller number of adults may have contributed to that year's run. This decline did not contribute to a poor adult return in 2005 when both N_e and census counts were the highest recorded in this study. More rigorous genetic analyses dedicated to looking at overlapping lineage relationships in these fish would help determine the rates of contribution from iteroparous individuals over time.

The research objectives of this study were to use new electronic tagging technologies to better describe the migratory characteristics of steelhead near the northern extent of their range and analyze the genetic population structure associated with various life histories of adult steelhead. In this study, acoustic and archival tags gave important information on steelhead kelts from the Ninilchik River migrating to and from the sea and while at sea between spawning migrations. Unique patterns of iteroparity and migratory behavior were observed. The scale and rate of iteroparity demonstrated by this study was similar to that found in other Alaskan streams, but did not support unique genetic population structure based on life history. These analyses suggest a panmictic population of steelhead on the Ninilchik River with a diversity of life history expressions that can vary over time. While this study revealed many heretofore undocumented aspects of anadromous steelhead kelts, many questions remain concerning the physical and biological factors contributing to life history structure and iteroparity in Alaskan steelhead. Fine-scale tag data on kelt movements, life history analyses, and genetics from this study suggest that steelhead have multiple migratory and reproductive phenotypes that contribute to reproductive success and population structure over time. Conservation and management of one or two reproductive phenotypes may not be sufficient in this complex species.

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Sea lice (*Lepeophtheirus salmonis*) infestations and the productivity of pink salmon (*Oncorhynchus gorbuscha*) in the Broughton Archipelago, British Columbia, Canada

Martin Krkošek and Ray Hilborn

Abstract: The spread of salmon lice (*Lepeophtheirus salmonis*) from salmon farms may threaten some wild salmon populations. Infestations of wild juvenile pink salmon (*Oncorhynchus gorbuscha*) have been associated with high mortality and population decline. Using stock–recruit data for pink salmon from the central coast of British Columbia, we analyzed how fishing mortality and spatial covariation combine with louse infestation to affect pink salmon population dynamics. The results indicate substantial coherence in survival at nested spatial scales — large-scale regional covariation and smaller scale covariation within management areas. Populations exposed to salmon farms (those from the Broughton Archipelago) show a sharp decline in productivity during sea lice infestations relative to pre-infestation years. Unexposed populations (comprising four management areas) did not experience a change in productivity during infestation years and had similar productivity to exposed populations before infestations. Our results suggest that sea lice infestations may result in declines of pink salmon populations and that management and policy of salmon farms should consider protecting wild juvenile salmon from exposure to sea lice.

Résumé : La dispersion de poux du saumon (*Lepeophtheirus salmonis*) à partir des élevages de saumons peut menacer certaines populations sauvages de saumons. Des infestations chez de jeunes saumons roses (*Oncorhynchus gorbuscha*) sauvages ont été associées à de fortes mortalités et des déclins de la population. À l'aide de données de stock-recrues de saumons roses de la côte centrale de la Colombie-Britannique, nous analysons de quelle manière la mortalité due à la pêche et la covariation spatiale se combinent à l'infestation de poux pour affecter la dynamique de population des saumons roses. Nos résultats indiquent une cohérence substantielle dans la survie à des échelles spatiales emboîtées — une covariation régionale à grande échelle et une covariation à plus petite échelle dans les aires de gestion. Les populations exposées aux élevages de saumons (celles de l'archipel de Broughton) subissent un net déclin de leur productivité durant les infestations de poux de mer par rapport aux années précédant l'infestation. Les populations non exposées (comprenant quatre aires de gestion) ne connaissent aucune modification de leur productivité durant les années d'infestation et ont une productivité semblable à celles des populations exposées avant les infestations. Nos résultats indiquent que les infestations de poux de mer peuvent causer des déclins des populations de saumons roses; la gestion et la politique des élevages de saumons devraient viser à protéger les jeunes saumons sauvages d'une exposition aux poux de mer.

[Traduit par la Rédaction]

Introduction

The growth of aquaculture (Duarte et al. 2007; Food and Agricultural Organization of the United Nations 2007; Naylor et al. 2000) has led to the emergence of infectious diseases that affect both fisheries and aquaculture (Gaughan 2001; Murray and Peeler 2005; Costello 2009). One example is the salmon louse (*Lepeophtheirus salmonis*), a marine ectoparasitic copepod that is native to the northern hemisphere and has become a threat to wild and farmed salmonids in Europe and Canada (Johnson et al. 2004; Costello 2006; Krkošek 2010*b*). Sea lice are common on both wild and farmed adult salmon and feed on host surface tissues causing morbidity and mortality at high infection intensity (Costello 2006; Pike and Wadsworth 1999). Sea lice infestations of wild juvenile salmonids during early marine life have been associated with reduced survival of some wild salmon populations (Gargan et al. 2002; Skilbrei and Wennevik 2006; Krkošek et al. 2007*a*). However, fisheries data are notoriously noisy, multifactorial, and subject to spurious correlation, leading to continued debate on the effects of sea lice on wild salmon survival (Brooks and Jones 2008; Rid-

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M. Krkošek[1,2] and R. Hilborn. School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195, USA.

[1]Corresponding author (e-mail: martin.krkosek@otago.ac.nz).
[2]Present address: Department of Zoology, University of Otago, 340 Great King Street, Dunedin 9016, New Zealand.

dell et al. 2008). For example, the links between louse transmission and salmon population dynamics may be mediated by predation (Krkošek et al. 2010), fish size (Jones and Hargreaves 2009), farm management (Morton et al. 2005), and the duration of exposure (Krkošek 2010*b*). In this paper, we evaluate how fishing mortality and environmental covariation combine with louse infestation to affect the productivity of pink salmon populations in the Broughton Archipelago, British Columbia.

Sea lice and salmon have been intensively studied in the Broughton Archipelago, particularly for pink salmon (*Oncorhynchus gorbuscha*) (Krkošek 2010*b*). Like other Pacific salmon, pink salmon are anadromous and semelparous, but they are also relatively unique among salmonids in life history. Unlike other salmon species (except chum salmon, *Oncorhynchus keta*), juvenile pink salmon do not rear in fresh waters but instead migrate to sea after hatching and then occupy nearshore (often intertidal) marine waters during a several-month period of rapid growth. After one winter, during which they migrate offshore, pink salmon then return to their natal rivers to spawn, thereby creating a life cycle that is 2 years long. Thus, there are distinct odd and even year populations that do not interbreed but yet occupy the same rivers. In terms of sea lice, much research has been focused on pink salmon because their small size at ocean entry makes them more vulnerable to infestation than larger salmon smolts. In addition, because pink salmon have a short life cycle (2 years) as opposed to other species, stock–recruit data have become available to test hypotheses about productivity and infestations for this species sooner than the others. For more information on pink salmon see Heard (1991).

Salmon farming began in the Broughton Archipelago in 1987 and has since grown in terms of the number of farms and overall production. Sea lice infestations of juvenile pink and chum salmon were first reported in the Broughton Archipelago in 2001 (Morton and Williams 2003) and have since occurred repeatedly there (Morton et al. 2004; Krkošek et al. 2005, 2006) and elsewhere in British Columbia (BC) (Morton et al. 2008). We consider infestations of pink salmon as beginning in the Broughton Archipelago in 2001 because they are conspicuous in nature and are unlikely to have gone previously unnoticed given the local population of biologists, first nations, and fishermen. The cause of a rapid (as opposed to gradual) increase in sea lice abundance may be related to host density thresholds, which can underlie the outbreak, as well as the eradication, of parasites in host populations (Krkošek 2010*a*). After the 2001 infestation, local populations of pink salmon collapsed, which generated recommendations from the Canadian Pacific Fisheries Resource Conservation Council to fallow the Broughton Archipelago to protect the juvenile salmon that were produced by the collapsed brood year (Pacific Fisheries Resource Conservation Council 2002). The recommendations were partially implemented by provincial regulators in spring 2003 that fallowed a primary migration corridor for juvenile pink salmon, which reduced sea lice abundance on juvenile salmon and improved the survival of that cohort (Morton et al. 2005; Beamish et al. 2006). However, infestations of juvenile pink salmon in the Broughton Archipelago occurred in 2001 (Morton and Williams 2003), 2002 (Morton et al. 2004), as well as 2004 and 2005 (Krkošek et al. 2006).

It is important to point out that sea lice are native to BC waters and have been present on wild salmon long before salmon farming began. In areas of BC without farms, the natural baseline estimates of louse infection of juvenile salmon during their first 3 months of marine life is approximately 5% (Krkošek 2010*b*; Krkošek et al. 2007*b*). Sea lice are rare on juvenile salmon during this time because the primary natural reservoir for lice, wild adult salmon, don't return to coastal waters on their spawning migration and overlap with wild juvenile salmon until summer when the juvenile salmon are larger and when transmission from wild adult to wild juvenile salmon occurs (Krkošek et al. 2007*b*; Krkošek 2010*b*). The working hypothesis is that transmission of sea lice from salmon farms to wild juvenile salmon can increase mortality of juvenile salmon via direct and indirect effects and thereby decrease productivity of wild salmon populations (Costello 2009; Krkošek 2010*b*). In this paper, we do not evaluate the effects of farms on sea lice transmission, as that has been done in numerous other works (Costello 2009; Krkošek 2010*b*). Rather, we test if the observed pattern of infestation is associated with expected declines in wild salmon productivity, while explicitly accounting for the potentially confounding effects of fishing mortality and spatial covariation inherent in pink salmon stock–recruit data.

Materials and methods

We used escapement data for pink salmon in one area exposed to salmon farms (the Broughton Archipelago) and a reference area not exposed to salmon farms (Fig. 1). The escapement data were described in two previous publications (Krkošek et al. 2007*a*; Ford and Myers 2008). Briefly, the data were collected primarily by stream walks and overhead flights by personnel at Fisheries and Oceans Canada. We screened the data, excluding populations with less than eight stock–recruit data pairs in the time series as well as rivers that experienced major changes in escapement methods or had enhancement projects. For example, the Klinaklini River in the Broughton Archipelago was excluded because escapement estimates changed from overhead flights to a fishwheel mark–recapture program. Exceptions include the Kakweiken River, which experienced a spawning channel that was largely unutilized by pink salmon, and the Glendale River, which also had a spawning channel constructed. For the Glendale River case, the spawning channel augmented production substantially, but we included this river nevertheless because of its numerical importance to the Broughton Archipelago stock complex. We compiled catch data from Fisheries and Oceans Canada for salmon management areas comprising the exposed (area 12) and unexposed populations (areas 7, 8, 9, 10). We assumed each population within an area experienced the same exploitation rate and modelled this with a random effect. For the 64 rivers in the unexposed area, there were 19 rivers in salmon management area 7, 28 rivers in area 8, 15 rivers in area 9, and 2 rivers in area 10 (Fig. 1). For these management areas comprising the unexposed populations, we also assumed each population within an area received the same exploitation rate.

The exposed and unexposed populations exist within a region of synchronous survival, suggesting shared environ-

Fig. 1. Map of the study area showing populations of pink salmon used in the analysis. There are populations from eight rivers categorized as exposed to salmon farms from the Broughton Archipelago (boxed region on the lower right). Unexposed populations are situated to the north and are identified by numbers 1–64. Populations are further grouped by Fisheries and Oceans Canada Management Areas 7 (rivers 1–19), 8 (rivers 20–47), 9 (rivers 48–62), 10 (rivers 63–64), and 12 (exposed populations). Salmon farms are identified by unnumbered small, black, filled circles. Squares and diamonds around farm symbols identify farms that were fallowed and held smolts, respectively, during the management intervention in 2003. Map was adapted from figure 1 in Krkošek et al. (2007*a*).

mental variation (Pyper et al. 2001). The populations also experience temporal autocorrelation, which can be accounted for in the Ricker model by parametric bootstrapping to obtain 95% confidence intervals on parameters (Dennis and Taper 1994). To account for the spatial and temporal correlation, we developed a mixed-model approach using the Ricker model, allowing for spatial synchrony in survival at regional and management area levels. We applied the model following a matched case-control experimental design in epidemiology, where subjects experiencing common environmental conditions are divided into control (unexposed populations) and treatment (exposed populations) groups,

and inference is conducted on differences between groups (Rothman 1986; Krkošek et al. 2008). The structure of the analysis is also similar to BACI (before–after control–impact) and IVRS (impact vs. reference sites) analyses (Wiens and Parker 1995), except that there is spatial and temporal replication within exposed and unexposed groups both before and during infestations and that statistical inference was conducted by comparing parameter estimates from a hierarchical Ricker model using a bootstrapping algorithm to generate 95% confidence intervals.

To structure the data according to a matched case-control study design, we divided the stock and recruitment data pairs into six categories: exposed and unexposed populations prior to sea lice infestations (recruitment 1972–2001), exposed and unexposed populations during sea lice infestations (recruitment between 2002 and 2006, except 2004), and exposed and unexposed populations during a fallowing management action (recruitment in 2004) corresponding to a management intervention (the provincial government's Sea Lice Action Plan: www.agf.gov.bc.ca/ahc/fish_health/sealice_BA_monitoring.htm) that fallowed farms along a primary wild juvenile salmon migration route and left remaining farms carrying primarily smolts (which are low infection risk) during the outmigration period of juvenile salmon in 2003 (Tribune Channel and Fife Sound; Fig. 1), which is the same cohort that returned to spawn in 2004 (Morton et al. 2005; Krkošek 2010*b*). For that year, juvenile pink salmon in the Broughton Archipelago encountered primarily fallowed farms (Kingcome, Wakeman, and Tribune populations) or fallowed and smolt farms (Knight Inlet populations). Because pink salmon have a 2-year life cycle, there are odd and even year lineages that occupy each river. Despite occupying the same habitats, the lineages are temporally separated and exhibit different genetics, abundances, and population dynamics (Heard 1991; Quinn 2005). We therefore treated each lineage as a separate population, as is common practice in other analyses of pink salmon stock–recruit data (Pyper et al. 2001; Dorner et al. 2008).

Stock–recruit model

The model development begins by describing the calculations for stock and recruitment values and then by building a stock–recruit model based on the Ricker model (Ricker 1954). We then build upon the basic Ricker model by including terms for the spatial structure of synchrony in salmon survival as well as considering variation in population growth rates in relation to spatial and temporal exposure to sea lice infestation. First, we incorporate fishing mortality to build the stock–recruit relationship for each population by calculating recruitment R for population i in year t as

$$(1) \qquad R_{i,t} = N_{i,t}(1-\mu_{i,t})^{-1}$$

where $N_{i,t}$ is the spawning abundance of pink salmon (i.e., escapement estimate) from population i in year t and $\mu_{i,t}$ is the exploitation rate (catch divided by catch plus escapement) for population i in year t. The spawning stock abundance for population i producing the recruitment in year t is simply the escapement estimate for that population but lagged by 2 years to account for the 2-year life cycle of pink salmon.

To formulate the base model, we assume stochastic Ricker population dynamics for pink salmon, giving the stock–recruit relationship

$$(2) \qquad R_{i,t} = N_{i,t-2}\exp(r - b_i N_{i,t-2} + \varepsilon_{i,t})$$

where r is the population growth rate, b_i determines density-dependent mortality and is related to the carrying capacity for population i, and $\varepsilon_{i,t}$ is a normally distributed random variable representing the influence of environmental stochasticity on pink salmon productivity. We do not consider measurement error in our modeling approach, but rather assume that it is small relative to the effects of environmental variation on salmon survival and that it contributes a small (but unknown) amount to the stochastic terms in the model. The process for fitting eq. 2 involves first transforming the model into its linear form

$$(3) \qquad \ln(R_{i,t}/N_{i,t-2}) = r - b_i N_{i,t-2} + \varepsilon_{i,t}$$

which can be fit by simple linear regression (Dennis and Taper 1994; Hilborn and Walters 2001).

With eq. 3 as the base, we developed and compared more complex models that structure components of environmental stochasticity to be coherent among populations within management areas and within the entire study region, as well as variation among populations exposed and not exposed to salmon farms and sea lice infestation. We first extended eq. 3 to include variation among populations exposed and unexposed to salmon farms before and during sea lice infestations

$$(4) \qquad \ln(R_{i,t}/N_{i,t-2}) = r_j - b_i N_{i,t-2} + \varepsilon_{i,t}$$

The index j corresponds to populations belonging to one of the six exposure categories — time periods before infestations, during infestations, and during the fallow treatment for populations exposed to salmon farms and populations not exposed to salmon farms (Fig. 1). Importantly, r_j are fixed effects in the following analysis as opposed to random effects, which we introduce next to account for the structure of variation (spatial coherence and among populations) in model parameters. We extended this model (eq. 4) by allowing for a spatially synchronous component in environmental stochasticity, as evidenced in Pyper et al. (2001), with a hierarchical or mixed modeling approach

$$(5) \qquad \ln(R_{i,t}/N_{i,t-2}) = (r_j + \theta_t) - b_i N_{i,t-2} + \varepsilon_{i,t}$$

Here, θ_t is a normally distributed random variable with mean equal to zero and variance to be estimated. Equation 5 contains two components to environmental stochasticity — variation among years synchronously for all populations (captured by θ_t) and variation among populations that is independent among years (captured by $\varepsilon_{i,t}$).

However, coherence among populations in environmental stochasticity may also occur at smaller spatial scales, with nearby populations exhibiting greater covariation in survival than distant populations (Pyper et al. 2001). For example, populations aggregated at the scale of Fisheries and Oceans Canada management areas within our study system may exhibit different patterns in population dynamics among the management areas. To accommodate this possibility, we ex-

tended eq. 5 to include a second hierarchical term for management area nested within year

$$(6) \quad \ln(R_{i,t}/N_{i,t-2}) = (r_j + \theta_t + \theta_{t,a}) - b_i N_{i,t-2} + \varepsilon_{i,t}$$

The hierarchical random effect components in eq. 6, θ_t and $\theta_{t,a}$, operate by structuring variation such that there is a random normal deviate (with mean equal to zero) common to all populations in each year t, and then another random normal deviation that further partitions variation within each year to populations according to which management area, a, they belong.

Finally, we also considered populations to have intrinsic variation among each other in r, and therefore included a third hierarchical component in the full model

$$(7) \quad \ln(R_{i,t}/N_{i,t-2}) = (r_j + \theta_i + \theta_t + \theta_{t,a}) - b_i N_{i,t-2} + \varepsilon_{i,t}$$

Here, the hierarchical random effects structure variation in a way similar to eq. 6 except that each population i has a population growth rate determined by a further random normal deviation from the growth rate r_j. The remaining variation structure in the hierarchical components follows similarly from eq. 6. In each year, the growth rate for each population has further random deviation from r_j, first according to the deviate for that year common to all populations and then according to the deviate for each management area a.

To complete the hierarchical model development and application, we considered all possible combinations of the random effects components in the hierarchical models. That is, we considered random effects on population, year (eq. 5), area, population plus year, population plus area, area within year (eq. 6), and population plus area within year (eq. 7). Although not all model formulations are detailed by equations in the main text, they are tabulated in the Results. For each formulation of random effects, we compared two models: one that included and one that excluded the fixed effects that structure the population growth rate by time periods preceding and during sea lice infestation (i.e., r is common to all populations in all years or r is specific to population group-period j). All models were fit to the data using maximum likelihood methods in the hierarchical modeling package lme4 in R (www.r-project.org). For each formulation of the random effects components, we used a likelihood ratio test to evaluate if including r_j in the model significantly improved the fit over the model with r constrained to the same value for all groups j. For the full set of candidate models, we used Akaike's information criteria to identify which model had the greatest support from the data. This process provided inference on which random effects components were important for explaining the data and whether structuring r according to population group-periods improved the model fit. However, it did not provide a statistical comparison among population growth rates from the different population groups of exposure to farms and infestation. To make statistical comparisons among these parameter estimates, we developed a bootstrapping algorithm to construct 95% confidence intervals on r_j as per Dennis and Taper (1994) (described in the next section).

We further evaluated the quality of fit of the best-supported model by analyzing the residuals and time series of random effects for covariation. We tested for temporal autocorrelation in the residuals, for both the entire data set as well as specifically for exposed populations, by using likelihood ratio tests to compare models for the mean of the residuals with and without a first-order autoregressive process (AR1). Similarly, we tested for temporal autocorrelation in the time series of random effects from the best-fit model by using likelihood ratio tests to compare models for the mean random effect with and without a first-order autoregressive process. Regardless of the significance of including the autoregressive process, we report the estimated correlation coefficient for each analysis. We also analyzed the residuals of the exposed populations for spatial covariation. To do this, we conducted pairwise Pearson correlation tests for each combination of exposed river pairs to evaluate covariation in residuals (positive or negative) for each possible pair of populations from rivers in the Broughton Archipelago. We also tested if there was significant correlation between the time series of exploitation estimates and time series of area-level random effects $\theta_{t,a}$ to evaluate if some of the area-level spatial covariation modeled in the data was due to assuming equal exploitation rates for each population within an area.

Growth rate comparisons

To statistically compare the estimates for r among exposed and unexposed populations during and before infestations, we used parametric bootstrapping to obtain 95% confidence intervals on r (Dennis and Taper 1994). However, the hierarchical structure of the data and models are more complex than analysis of individual time series as in Dennis and Taper (1994). We therefore modified the bootstrapping approach in a way that accounted for the hierarchical spatial structure in the data, exploitation, missing data values, and changes in productivity according to louse infestations. The bootstrapping algorithm forecasted recruitment values for each $N_{i,t}$ observation where there was a valid observed $N_{i,t}$ and $N_{i,t-2}$ data pair. The value of r used for each forecasted value corresponded to the estimate from unexposed and exposed populations before and during infestations. To include stochasticity in the simulation, we followed the same hierarchical structure in the hierarchical Ricker models that were fit to the stock–recruit data, of which eq. 6 was the best supported model (see Results).

To use eq. 6 in the bootstrap algorithm, we first drew a random deviate from θ_t for each year. Within each year we drew five random deviates — one for each management area in each year — which were then applied to each population in the respective management area. Finally, residual random environmental variation, represented by $\varepsilon_{i,t}$, was applied by drawing a random variable from $\varepsilon_{i,t}$ separately for each population in each year. With the stochastic components of eq. 6 generated appropriately, we then used the untransformed version of eq. 6

$$(8) \quad R_{i,t} = N_{i,t-2}\exp[(r_j + \theta_t + \theta_{t,a}) - b_i N_{i,t-2} + \varepsilon_{i,t}]$$

to simulate recruitment data for each $N_i(t)$ observation where there was a valid observed $N_{i,t}$ and $N_{i,t-2}$ data pair. We then fit the same model (eq. 6) to the simulated data in exactly the same manner as with the original data and recorded the values of r for each population group (before infestations, during infestations, and the fallow year for exposed and un-

exposed populations). This bootstrap procedure was repeated 1000 times, generating six sets of 1000 estimates for r corresponding to unexposed and exposed populations preceding infestations, during infestations, and during the fallow. Each of the 95% confidence intervals on r then correspond to the 2.5 and 97.5 percentiles of the 1000 bootstrap estimates.

We also evaluated the potential effects of measurement error on uncertainty in the parameter estimates by repeating the above simulations under scenarios where the standard deviation of the residual error was divided between process error and measurement error. To evaluate the potential effects of measurement error, we assumed a log-normal distribution of uncertainty around the escapement estimates and then multiplied each escapement estimate by a random number drawn from the log-normal distribution to determine the number of spawners used in the forecast of recruits. The log-normal distribution had a mean of zero and a standard deviation that was a proportion of the residual error. We conducted four simulations, where measurement error had standard deviations that were 0%, 25%, 50%, and 75% of the standard deviation of the residual errors. The process error in these simulations had corresponding reductions in standard deviation such that 100%, 75%, 50%, and 25% of the standard deviation of the residual error was allocated to process error $\varepsilon_{i,t}$.

Results

The time series of escapement data for pink salmon in the study region indicates populations in the exposed area experienced a decline in abundance relative to populations from unexposed areas during the period of sea lice infestations (Fig. 2). During infestation years, escapement to the exposed populations declined (except for the fallow year) while at the same time there was a reduction in fishing mortality from rates commonly exceeding 50% to rates less than 10% (Fig. 2). Unexposed populations also experienced a decline in fishing mortality in recent years, with populations in management areas 9 and 10 (rivers 48–64 in Fig. 1) experiencing zero fishing mortality in recent years (Fig. 2). However, the majority of populations in the unexposed area were from management areas 7 and 8 (rivers 1–47), where exploitation rates remained between 10% and 40% during infestation years. Although exposed populations in the Broughton Archipelago experienced exploitation between 5% and 10% during infestation years, the targeted fisheries on these populations have been closed, and the estimated fishing mortality is mostly due to incidental mortality from fisheries targeting more southern populations as they migrate through Queen Charlotte Strait and Johnstone Strait (Fig. 1). Despite these changes in fishing mortality, exposed populations experienced a marked decline in survival during sea lice infestation years, whereas unexposed populations did not (Table 1; Fig. 2).

We fit and compared many variations of the Ricker model to the data (Table 2). The model formulations differed in two particular ways: (*i*) the structure of hierarchical random effects terms and (*ii*) whether the population growth rate was fixed to the same value for all populations or was structured by exposure to salmon farms and sea lice infestations. For each formulation of random effects, likelihood ratio tests indicated that structuring the population growth rate according to sea lice infestation and exposure to salmon farms significantly improved the fit of the model (Table 2). That is, the null hypothesis that the population growth rate was equal among populations exposed and not exposed to salmon farms before and during sea lice infestations was rejected. Among the various model formulations, likelihood values and Akaike's information criteria indicated that the model with hierarchical random effects for year and management area within year was best supported by the data. This suggests populations show spatial covariation in survival rates throughout the study area and also within management areas. The results also indicate there was no evidence for variation in average productivity among populations (θ_i) or areas (θ_a) (Table 2). The best model (eq. 6, Table 2) showed substantial variation in the components of stochasticity, with the standard deviation for regional coherence among years (the random effect on year) equal to 0.61, the standard deviation for coherence within management area (the random effect for area within year) equal to 0.50, and residual standard deviation equal to 1.12.

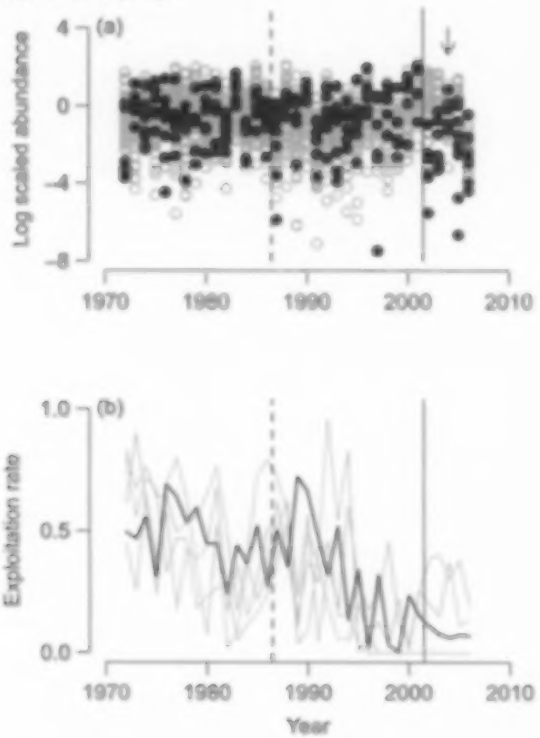


Fig. 2. Time series of scaled escapement estimates ($\ln(N_{i,t}/m_i)$, where m_i is the mean escapement for population i) and exploitation rates (proportion of returning salmon harvested by fisheries) for unexposed populations (grey points and lines) and exposed populations (black points and lines). Escapement data are given per river, whereas exploitation rates are given per management area. The vertical dotted line marks the onset of salmon aquaculture in the Broughton Archipelago, the vertical thin line marks the onset of sea lice infestations, and the arrow identifies the fallow treatment in the Broughton Archipelago.

Table 1. Observations of log recruits per spawner ($\ln(R_t/N_{t-2})$) for pink salmon populations during sea lice infestation years and the fallow treatment (2004).

Population	2002	2003	2004	2005	2006
Ahta	–4.363	–3.088	3.459	1.129	–2.657
Kakweiken	–4.916	–1.679	2.520	0.646	–0.478
Kingcome	–3.680	–0.999	2.691	–1.864	–1.863
Wakeman	–1.879	1.097	0.927	–3.580	–1.484
Viner	–0.396	–2.020	1.632	–0.979	–2.953
Lull	–6.616	–5.234	4.345	3.626	–3.089
Ahnuhati	–4.000	0.478	2.885	0.059	–2.471
Glendale	–3.617	–2.044	3.656	0.396	–1.220
Average	–3.683	–1.686	2.764	–0.071	–2.027

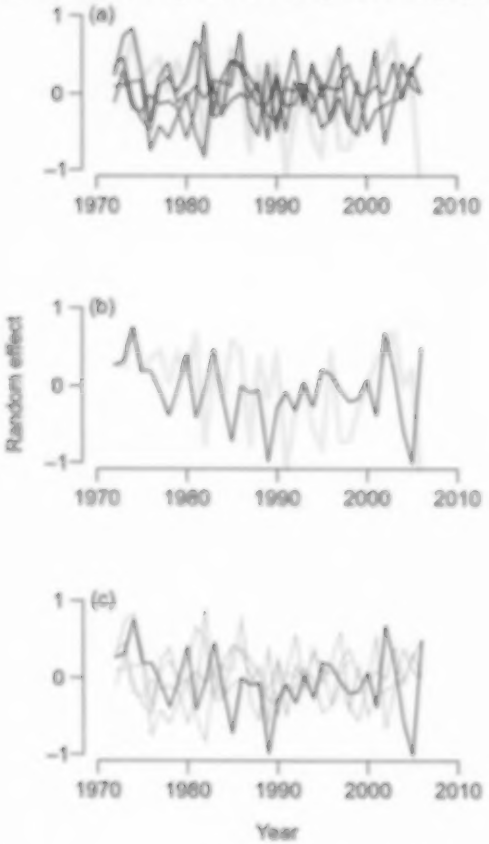
Table 2. Results of fitting various formulations of the Ricker model.

Random effects	Lice	NLL	AIC	ΔAIC	χ^2	df	p
None	No	2866.8	5973.6	460.5			
	Yes	2820.3	5890.7	377.6	93	5	2.20×10^{-16}
P	No	2866.8	5975.6	462.5			
	Yes	2820.3	5892.7	379.6	93	5	2.20×10^{-16}
Y	No	2709.1	5660.3	147.2			
	Yes	2673.6	5599.2	86.1	71.1	5	5.97×10^{-14}
A	No	2866.8	5975.6	462.5			
	Yes	2820.3	5892.7	379.6	93	5	2.20×10^{-16}
P + *Y*	No	2709.1	5662.3	149.2			
	Yes	2673.6	5601.2	88.1	71.1	5	5.97×10^{-14}
P + *A*	No	2866.8	5977.6	464.5			
	Yes	2820.3	5894.7	381.6	93	5	2.20×10^{-16}
Y/*A*	No	2646.6	5537.1	24			
	Yes	**2629.6**	**5513.1**	**0**	**34**	**5**	$\mathbf{2.37\times10^{-6}}$
P + *Y*/*A*	No	2646.6	5539.1	26			
	Yes	2629.6	5515.1	2	34	5	2.37×10^{-6}

Note: Random effects indicates the nesting structure of random effects components in the hierarchical Ricker model, with *P* indicating population, *Y* indicating year, and *A* indicating area. For example, *P* + *Y*/*A* indicates that area was nested within year and that population was a separate random effect. The column Lice indicates if the population growth rate r was structured according to periods preceding infestation, during infestation, and during the fallow treatment for exposed and unexposed populations. A value of "no" in the Lice column corresponds to a null model formulation where r was a single parameter common to all population groups. For each formulation of random effects, we give the likelihood ratio statistic (χ^2) and associated degrees of freedom (df) and p value from the likelihood ratio test comparing models with and without r structured by infestation and exposure information. The likelihood ratio tests each had five degrees of freedom — the difference in the number of parameters between the candidate models. Other columns show the negative log-likelihood value (NLL) as well as the Akaike information criteria (AIC) and AIC differences (ΔAIC) for each model. The best-supported model is indicted in bold.

Analysis of the best-fit model indicated that the time series of random deviations for the year and area within year random effects showed substantial environmental stochasticity organized by regional and management area scales (Fig. 3). There was low-level, negative temporal autocorrelation in the residuals for the full data set but no systematic temporal autocorrelation in the random effects from the best-fit model (eq. 6), aside from weak temporal autocorrelation in the random effects for area 9 (Table 3). There was no temporal autocorrelation detectable in the residuals for the exposed populations (Table 3 and Fig. 4), but there was a small degree of spatial covariation evident among rivers in the exposed region (Table 4). Of the 28 pairwise comparisons, nine showed significant correlation, of which six were negative correlations and three were positive correlations. Much of the spatial covariation is attributable to pink salmon from the Glendale River, which covaried significantly with four of the seven populations. Interestingly, the structure of covariation with the Glendale population involved positive correlation with rivers nearby, no correlation with rivers at intermediate distance, and negative correlation with more distant rivers within the Broughton Archipelago (Table 4 and Fig. 1). The time series of area-level random effects, $\theta_{1,a}$, were not significantly correlated with exploita-

Fig. 3. Time series of hierarchical random effects components, θ, of the best-fit model (eq. 6). The random deviate common to all populations for each year θ_t is shown as the thick grey line in panels (*a*) and (*b*). The random deviates for each unexposed area $\theta_{t,a}$ (management areas 7, 8, 9, 10) within each year are shown as the four black lines in panel (*a*). The random deviates for the exposed area within each year $\theta_{t,a}$ are shown as the black line in panel (*b*). Panel (*c*) shows the time series of random deviates within each year $\theta_{t,a}$ for the exposed area (black line) relative to the random deviates within each year for the unexposed areas (grey lines).



tion rates for management areas 8, 9, and 12, but were significantly correlated for management areas 7 and 10 (Table 5). Introducing measurement error (ME) into the bootstrap procedure had small effects on the estimated 95% confidence intervals for the population growth rate of exposed populations during infestation years (0% ME: –1.72 to –0.32; 25% ME: –1.68 to –0.27; 50% ME: –1.73 to –0.30; 75% ME: –1.74 to –0.31).

Model selection statistics (Table 2) as well as estimates of the population growth rates (r; Table 6 and Fig. 5) indicate that r was not equal among data groups. To statistically compare estimates of r among data groups, we turned to a parametric bootstrapping algorithm to construct 95% confidence intervals on r for stock–recruit data grouped by exposure to salmon farms and sea lice infestation. The results of the bootstrapping procedure indicate that exposed and unexposed populations did not differ in productivity preceding sea lice infestations and that during sea lice infestations productivity of exposed populations declined significantly relative to pre-infestation years. In contrast, unexposed populations did not experience a change in productivity during sea lice infestations (Table 6, Fig. 5). During the fallow year, exposed populations experienced an increase in productivity, whereas unexposed populations did not change substantially (Table 6, Fig. 5), although there were few data and no temporal replication for the fallow treatment.

Discussion

Our analysis of stock–recruit data for pink salmon evaluated how the effects of fishing mortality and spatial hierarchies of environmental covariation combine with louse infestation to affect the productivity of pink salmon populations. Diagnostic analysis of the best-fit model indicates there was little covariation remaining in the random effects and residuals of the model and that measurement error likely has little effect on uncertainty in parameter estimates. Overall, the small amount of covariation in residuals and random effects is unlikely to affect the results, which indicate that there is substantial natural variation among and within pink salmon populations and that louse infestations can depress and extirpate pink salmon populations. These results are consistent with previous predictions of high mortality of pink salmon during infestations (Morton and Routledge 2005; Krkošek et al. 2006; Krkošek et al. 2010), analyses of population dynamics of pink salmon (Krkošek et al. 2007*a*) and coho salmon (Connors et al. 2010) in relation to sea lice infestations, and the global pattern of decline of wild salmon stocks exposed to industrial salmon aquaculture operations (Ford and Myers 2008).

For pink salmon, spatial covariation in survival decays with distance between stocks, but can still persist at regional scales encompassing 500–1000 km (Pyper et al. 2001, 2005). The spatial scale that encompasses the pink salmon populations in our study (~500 km) indicates we should expect a small degree of regional coherence in survival rates as well as a larger degree of local coherence in survival rates, possibly due to local environmental conditions as well as fishing mortality. The model accommodated these two spatial scales of covariation in survival via a random effect on year for all stocks collectively and also separately for stocks within separate management areas. This also accounted for any synchronizing effect at the management area scale that could have been caused by the assumption of equal exploitation rates among populations within a management area. However, the analysis of random effects indicated two significant correlations (out of five possible) between area-level random effects and harvest rates, indicating our treatment of exploitation rates did not substantially confound natural coherence at the spatial scale of management units. The diagnostic analysis of the best-fit model found there was minimal spatial covariation among populations in the residuals, indicating our treatment of individual populations as independent samples was robust within the modeling framework. The hierarchical model was further able to differentiate natural regional variability from sea lice effects, such as in 2006 when there was a large negative random effect on all populations that contributed to the poor survival observed during sea lice infestation of exposed populations.

Table 3. Tests for temporal autocorrelation in residuals and random effects in the best-fit model (eq. 6).

	NLL (null)	NLL (alt.)	L ratio	p	Φ
Residuals (all)*	2457.19	2441.88	30.62	<0.0001	–0.14
Residuals (BA)	408.48	408.39	0.18	0.67	0.03
θ_t Year	29.83	29.82	0.01	0.91	0.02
$\theta_{t,a}$ Area 7	16.57	15.36	2.41	0.12	0.27
$\theta_{t,a}$ Area 8	17.38	17.28	0.19	0.66	–0.02
$\theta_{t,a}$ Area 9	14.70	12.81	3.78	0.05	0.36
$\theta_{t,a}$ Area 10	4.75	4.17	1.17	0.28	0.28
$\theta_{t,a}$ Area 12	17.19	17.11	0.15	0.70	–0.07

Note: Residuals are analyzed for the entire data set (all) as well as for the exposed populations in the Broughton Archipelago (BA), specifically. Time series for random effects occurred for an overall year effect (θ_t) as well as for each area ($\theta_{t,a}$; areas 7, 8, 9, 10, 12). Likelihood ratio tests were conducted by comparing models for the mean residual or random effect versus models for the mean residual or random effect with a first-order autoregressive process (see Materials and methods). Shown are the negative log-likelihood values (NLL) for null models (null; no temporal autocorrelation) and alternative models (alt.; first-order autoregressive process), the likelihood ratio test comparing null and alternative models (L ratio), corresponding p values, and the estimated coefficient for temporal autocorrelation (Φ). Significant effects ($\rho = 0.05$) for temporal autocorrelation are shown by an asterisk (*).

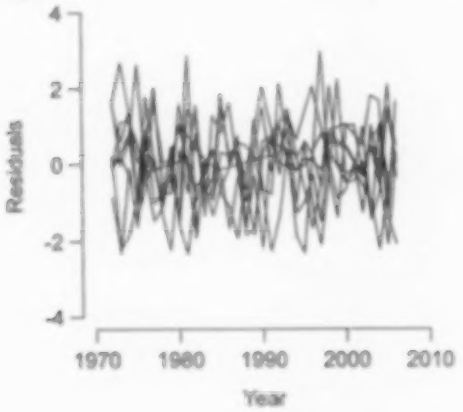


Fig. 4. Time series of residuals from the best-fit model (eq. 6) for populations from each river in the exposed region, the Broughton Archipelago.

There was, however, one interesting result from the analysis of the spatial covariation of residuals. Of the nine significant correlations of residuals among populations, four were associated with the Glendale River, which covaried with four of the seven other populations. Correlations with Glendale River were positive for nearby rivers and negative for more distant rivers. Because Glendale River has experienced major enhancement (via a spawning channel built in the 1990s) and is numerically dominant among populations in the Broughton Archipelago, this result has interesting implications. One interpretation is that enhancement in one large river may have positive effects on nearby rivers (possibly because of straying) and negative effects on more distant rivers (via competition) where straying rates are reduced but with which there is significant spatial overlap in juvenile salmon habitats. Because enhanced fish are not distinguished from native fish in rivers near Glendale River, it is not possible to evaluate if the positive correlation is indeed due to straying nor is it possible to evaluate if native populations in nearby rivers (i.e., the subset of fish that are not strays from the spawning channel in Glendale) actually experience a decline in survival due to increased competition. Nevertheless these results are consistent with other findings that enhancement may have negative effects on nearby populations via competition (Hilborn and Eggers 2000), which has also been posited by other authors as an alternate hypothesis for declines in pink salmon populations in the Broughton Archipelago (Riddell et al. 2008). Our results indicate that effects of enhancement in Glendale River may indeed be a component of pink salmon population dynamics in the Broughton Archipelago, but such effects are in addition to — rather than in place of — the effects of louse infestation. During infestations, Glendale River populations followed the same pattern of decline as the other populations in the Broughton Archipelago (Krkošek et al. 2008).

By incorporating fishing mortality, we have better captured the impact of infestations on wild salmon productivity relative to previous estimates, which did not consider variation in harvest rates (Krkošek et al. 2007*a*). Fishing mortality is a potential confounding factor, since exploitation rates on pink salmon from the Broughton Archipelago have varied from 0.2% to 72% within the time series. The estimated impact of infestations in our analyses was a decline in the population growth rate of pink salmon by 2.15 from $r = 1.15$ prior to infestations to $r = -1.0$ during infestations, a magnitude that is 16% larger than that proposed by Krkošek et al. (2007*a*). In addition, the uncertainty in parameter estimates was robust to measurement error, as shown by the bootstrap procedure. However, future analyses might consider state–space models as a way of formally modeling both process and measurement error in one analysis. However, such analyses may not control for possible systematic bias in measurement error. For example, the exceptional high survival for the 2004 brood year (Beamish et al. 2006) may be partially due to increased search effort in spawner counts as motivated by population collapse in the previous 2 years. The current analysis indicates the size of the 95%

Table 4. Pairwise Pearson correlations for time series of residuals from the best-fit model (eq. 6) for each river in the Broughton Archipelago.

	AT	KK	KC	WM	VN	LL	AN	GD
AT	—	0.665	0.207	0.308	0.406	0.950	0.053	0.260
KK	–0.076	—	0.915	0.973	0.015*	0.708	0.019*	0.123
KC	–0.226	0.019	—	0.629	0.729	0.170	0.368	0.005*
WM	–0.183	–0.006	0.087	—	0.230	0.005*	0.725	0.035*
VN	0.186	–0.510*	–0.083	0.281	—	0.033*	0.002*	0.117
LL	–0.011	0.068	–0.253	–0.489*	–0.456*	—	0.675	0.022*
AN	–0.340	0.407*	–0.162	–0.064	–0.658*	0.078	—	0.013*
GD	–0.196	0.266	–0.476*	–0.369*	–0.344	0.397*	0.427*	—

Note: The p values for pairwise correlations are shown above the diagonal (upper right) and the correlation coefficients are shown below the diagonal (lower left). Significant ($p < 0.05$) data are indicated with an asterisk (*). River identities are Ahta (AT), Kakweiken (KK), Kingcome (KC), Wakeman (WM), Viner (VN), Lull (LL), Ahnuhati (AN), and Glendale (GD).

Table 5. Results of Pearson product moment correlation tests between time series of exploitation rates and area-level random effects $\theta_{i,a}$ for each management area.

Area	t	df	p	Correlation	95% CI
7	4.17	33	0.0002	0.59	(0.32 to 0.77)
8	0.63	33	0.53	0.11	(–0.23 to 0.42)
9	1.15	33	0.26	0.20	(–0.15 to 0.50)
10	2.10	28	0.04	0.37	(0.01 to 0.64)
12	–0.38	33	0.71	–0.07	(–0.39 to 0.27)

Note: Data shown include the test statistics (t statistic, degrees of freedom (df), p values) and estimated correlation with the 95% confidence interval (CI) for each management area.

confidence intervals on the estimates of the population growth rate is larger than that of Krkošek et al. (2007*a*), reflecting the increased difficulty in measuring the effects of sea lice infestation given the spatial hierarchical structure in survival combined with the relatively low level of temporal replication within the sea lice infestation category (4 years).

The change in productivity of pink salmon populations is from a recruits-per-spawner value of 3.16 prior to infestations compared with a recruits-per-spawner value of 0.37, which corresponds to a decline in survival by 90%. This change in survival is consistent with similar trends for declines in coho salmon productivity in the Broughton Archipelago associated with sea lice infestations (Connors et al. 2010), estimates of direct mortality of juvenile salmon during infestations (Morton and Routledge 2005; Krkošek et al. 2006) plus indirect effects via mortality from predators (Connors et al. 2008; Krkošek et al. 2010). However, it is important to point out, as others have (Brooks and Jones 2008), that experimental infection studies consistently show high survival of juvenile salmon and high mortality of lice, suggesting parasite rejection of juvenile salmon that are larger than 0.7 g (Jones et al. 2006, 2007; Jones and Hargreaves 2009). The differences in mortality suggested by field and experimental studies may be reconciled by the duration of exposure to sea lice, which can be 2–3 months in field conditions as opposed to less than 1 day in experimental studies (Krkošek et al. 2009), or may be due to increased predation risk of infected fish (Krkošek et al. 2010). The negative population growth rate for infested populations implies population decline (Dennis et al. 1991; Lande 1993) if the environmental conditions causing the decline are sustained. However, we note that a new management system has been implemented in the Broughton Archipelago that combines fallowing and chemical treatment to minimize the risk of sea lice transmission from farmed salmon to wild juvenile salmon. Evaluation of this management plan for reducing sea lice and halting the decline is an important area of current research (Morton et al. 2011).

One key assumption in the analysis is that sea lice infestations of juvenile wild pink salmon in the Broughton Archipelago began in 2001. Morton and Williams (2003) reported high levels of lice on juvenile wild pink salmon in the Broughton Archipelago in 2001, with prevalence of infection exceeding 98% and intensity of infection exceeding 12 lice per fish at locations exposed to farms. Infestations on this scale are highly conspicuous to casual observation from docks or boats without a need for collection and examination of fish to be noticed. Given the local population of biologists, fishermen, fish farmers, and First Nations peoples in the Broughton Archipelago, we consider it unlikely that infestations of this scale occurred without notice prior to 2001. The sudden emergence of infestations is a common characteristic of host–parasite ecology due to the effects of host-density thresholds, where parasite population dynamics can switch from endemic to outbreak conditions (Grenfell and Dobson 1995). Such effects have occurred for sea lice on salmon farms in other regions (New Brunswick, Canada) and are likely to occur in aquaculture–fishery interactions because of increases in aquaculture production, changes in abiotic factors that favour parasite population growth (e.g., warming), or increases in the abundance of sympatric wild host populations (Krkošek 2010*a*). Given gradual trends in ocean warming, increases in aquaculture production, and high return abundances of adult pink salmon in 2000, it may be that these factors combined to trigger outbreak conditions for sea lice. Nevertheless, the absence of sea lice data from years preceding 2001 means we are unable to test the assumption that infestations of sea lice began in the Broughton Archipelago in 2001.

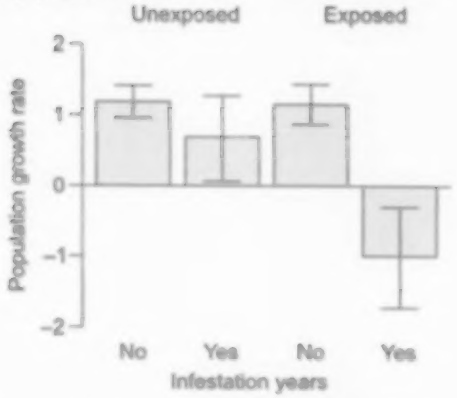
The productivity of fish stocks at low population size, also known as the maximum reproductive rate or the population growth rate, is fundamental to understanding the population biology of fish and informing fisheries management (Myers et al. 1995, 1999). The population growth rate for pink salmon populations not subjected to sea lice infesta-

Table 6. Population growth rates (r) with 95% confidence intervals (CI) for pink salmon populations exposed and unexposed to salmon farms before and during sea lice infestations.

	Before infestations	During infestations	Fallow
Exposed	1.15 (n = 219; CI: 0.87 to 1.43)	−1.00 (n = 32; CI: −1.72 to −0.32)	2.81 (n = 8; CI: 1.36 to 4.24)
Unexposed	1.19 (n = 1259; CI: 0.96 to 1.42)	0.70 (n = 102; CI: 0.08 to 1.31)	0.18 (n = 25; CI: −1.12 to 1.46)

Note: Data in parentheses are point estimates for r from analyzing the unscaled escapement data and the sample sizes (n), the number of stock–recruit data pairs in each category.

Fig. 5. Population growth rates (±95% confidence intervals, CI) for pink salmon populations from exposed and unexposed regions prior to and during years when sea lice infestations were observed for exposed populations.



tions in our analysis was very near the expected value of r = 1.2 (standard deviation = 0.35) estimated for pink salmon in the large meta-analysis by Myers et al. (1999). Similarly, analyses of stock–recruit time series of pink salmon from numerous stocks from BC, Washington, and Alaska indicate good agreement between our estimates of natural productivity and those from the BC central coast as well as the spatial variation in population growth rates (Su et al. 2004; Dorner et al. 2008). This indicates that for populations not experiencing sea lice infestation, the productivity of pink salmon populations in the study region was characteristic of basic pink salmon biology elsewhere throughout their range. However, one departure from the other multistock analyses of pink salmon (e.g., Dorner et al. 2008) is that there was little indication for variation in average productivity among populations or areas (i.e., random effect on population or area). One explanation may be that other multistock analyses cover large spatial variation in stocks (from Washington through Alaska), which would imply greater variation among populations in average productivity due to habitat and climate variation. The populations we studied were at a spatial scale that is smaller than an individual stock in the other analyses, meaning that spatial variation in climate and habitat would be relatively low.

The years of infestations of juvenile wild pink salmon spanned 2001 to 2005 (Morton and Williams 2003; Morton et al. 2004; Krkošek et al. 2006) but also contained a management intervention in 2003 where farms on the migrations routes were primarily fallowed or carried smolts (Morton et al. 2005). Sea lice abundances on juvenile wild salmon in the Broughton Archipelago in 2003 were markedly reduced relative to infestation years (Morton et al. 2004, 2005; Jones and Hargreaves 2007). Our analysis here agrees with other analyses that the 2003 cohort that experienced the fallow treatment and returned to spawn in 2004 also experienced excellent marine survival (Beamish et al. 2006; Krkošek et al. 2007*a*). However, the increased productivity of this cohort (r estimated as 2.8) greatly exceeds natural rates (population growth rate is typically 1.2, as discussed above), which indicates other factors in the marine environment in addition to reductions in sea lice abundances likely contributed to the productivity of this cohort. However, we caution against overinterpretation of the effects of the fallowing intervention in this analysis, as it only occurred in one year and has not been replicated. Carefully controlled replication of fallowing and (or) chemical treatment is required before sufficient data are accumulated to evaluate the efficacy of management interventions to reduce sea lice and protect wild pink salmon in the Broughton Archipelago. Such a management system is now in place and is an important area of focus for current work (Morton et al. 2011).

Our study design, which compared exposed and unexposed populations before the infestations, during the infestations, and during the fallow, is related to a matched case-control statistical design used in epidemiology (Rothman 1986). The reasoning is that after conditioning on a shared environment, differences between subjects that received a treatment and subjects that did not are attributable to a treatment effect — sea lice infestation in our case. An unavoidable drawback of applying this approach to field data is that control (unexposed populations) and treatment (exposed populations) are not assigned randomly but are based on observations of proximity to salmon farms and sea lice infestations (Krkošek et al. 2008). Further, although the changes in louse abundance for the exposed group may resemble a "natural experiment", this does not constitute an intentional manipulation to test a hypothesis, making results more difficult to interpret than their experimental counterparts (Lubchenco and Real 1991). For sea lice and salmon, alternate factors cannot be ruled out, but no confounding factors have been identified by regulatory agencies or scientists despite millions of dollars invested into several multiyear studies (Fraser et al. 2009). Nevertheless, a large-scale experiment where sea lice abundances are manipulated according to the scientific principles of randomization and replication would help determine causation between sea lice and salmon productivity. While our results indicate the spread of infection from salmon farms may threaten wild pink salmon populations in the Broughton Archipelago, further work is needed to expand the spatial and taxonomic sample size of studies on these effects. Nevertheless, our results suggest that sea lice infestation is likely a factor that influences the productivity of wild salmon.

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Estimating larval production of a broadcast spawner: the influence of density, aggregation, and the fertilization Allee effect

Carolyn J. Lundquist and Louis W. Botsford

Abstract: The effect of fishing on reproduction is typically quantified by computing the effects of changes in the species abundance and age structure on egg production. For broadcast spawners, reproduction also depends on the local spatial distribution of individuals. Broadcast spawners exhibit an Allee effect at low density: a decline in the fertilization of eggs, owing to increased distance between spawners. We present a method for assessing the likely impact of a fishery on broadcast spawners, based on gamete dispersion dynamics and individual spatial distributions. We use an individual-based model to simulate larval production over a range of uncertainties in dispersion characteristics. We illustrate our method for the red sea urchin, *Strongylocentrotus franciscanus*, fishery in northern California, USA. The density of red sea urchins varied over space (0.1–1.6·m^{-2}), and indices of aggregation were highest at low densities. As gamete dispersion distances increased, larval production exhibited a more linear relationship with density. Average larval production in 1996–1998 was 33.8% of production near the inception of the fishery. After accounting for decreases in mean density, the fertilization Allee effect accounted for 21.7 ± 4.1% of the decrease in larval production, and 45.2 ± 21.7% if sea urchins were not aggregated.

Résumé : On mesure normalement l'effet de la pêche sur la reproduction en calculant les conséquences des changements de l'abondance et de la structure en âge sur la production d'œufs. Pour les reproducteurs à la volée, la reproduction dépend aussi de la répartition spatiale locale des individus. À faible densité, les reproducteurs à la volée subissent aussi un effet d'Allee, un déclin de la fécondation des œufs à cause de la distance accrue entre les reproducteurs. Nous présentons une méthode pour évaluer l'impact vraisemblable de la pêche sur les reproducteurs à la volée basée sur la dynamique de la dispersion des gamètes et les répartitions spatiales individuelles. Nous utilisons un modèle basé sur l'individu pour simuler la production larvaire sur une gamme d'incertitudes dans les caractéristiques de la dispersion. Nous illustrons notre méthode avec une pêche de l'oursin rouge géant, *Strongylocentrotus franciscanus*, dans le nord de la Californie, É.-U. La densité varie en fonction de l'espace (0,1–1,6·m^{-2}) et les indices de regroupement sont maximaux aux faibles densités. À mesure que les distances de dispersion des gamètes augmentent, la production larvaire montre une relation plus linéaire avec la densité. La production larvaire moyenne en 1996–1998 représentait 33,8 % de la production observée vers le début de la pêche commerciale. Après avoir tenu compte des diminutions de la densité moyenne, l'effet d'Allee sur la fécondation explique 21,7 ± 4,1 % du déclin de la production larvaire et 45,2 ± 21,7 % lorsque les oursins ne sont pas regroupés.

[Traduit par la Rédaction]

Introduction

Reduced density due to fishing can impact the population dynamics of fished species in many ways. Fishery models describing stock–recruitment relationships most often assume that while density-dependence may be compensatory at high density, it is linear at low density. However, some species exhibit an Allee effect at low levels of abundance, such that recruitment is severely reduced to levels below that which a linear decline would predict (Courchamp et al. 1999). Many mechanisms can lead to Allee effects (see, e.g., Courchamp et al. (1999), but here we are concerned with decreased fertilization efficiency at low spawning densities (reviewed in Levitan (1995)). Such Allee effects are suspected to underlie the failure of some benthic invertebrates to recover after fishing pressure is removed (e.g., white abalone in southern California, Tegner et al. (1996)).

To account for the effect of fishing on reproduction, stock assessment scientists commonly compute the effects of a narrowed age structure and reduced population density on

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C.J. Lundquist.[1] National Institute of Water and Atmospheric Research, Ltd., P.O. Box 11115, Hamilton 3251, New Zealand; Bodega Marine Laboratory, P.O. Box 247, Bodega Bay, CA 94923, USA.
L.W. Botsford. Bodega Marine Laboratory, P.O. Box 247, Bodega Bay, CA 94923, USA; Department of Wildlife, Fish, and Conservation Biology, University of California – Davis, Davis, CA 95616, USA.

[1]Corresponding author (e-mail: c.lundquist@niwa.co.nz).

egg production. The effects of egg production on population dynamics are described through a nonlinear, compensatory dependence of recruitment on egg production (the latter may be approximated by spawning stock biomass). Some fished species, including sea urchins and abalone, are broadcast spawners, which release gametes directly into the water column. For these species, rates of external fertilization are influenced by distance between spawning individuals and hydrodynamic conditions that influence the distance and direction of gamete dispersal. For broadcast spawners, effective reproduction (i.e., larval production) also depends on the effect of fishing on the local spatial distribution of individuals. In this case, the reduction in density due to fishing can lead to a decline in the fraction of eggs fertilized, which may cause a depensatory density-dependent effect of recruitment on egg production.

The specific effect of a decline in population density on larval production in broadcast spawners depends on (*i*) the pattern of gamete dispersion and (*ii*) the resulting spatial distribution of individuals, in addition to the effect of fishing on age or size structure (Lundquist and Botsford 2004). A sharp threshold decline in larval production would not occur except in biologically unrealistic situations (disk-shaped gamete dispersion and evenly spaced individuals) (Lundquist and Botsford 2004). For random and aggregated distributions of individuals, as gamete dispersion distance decreases, dynamics change from a clearly defined Allee effect at low densities to an approximately linear relationship over the complete range of densities (Lundquist and Botsford 2004) (Fig. 1; for clarity the additional effects of other compensatory mechanisms at higher density are not shown here).

One problem faced when attempting to account for the effects of fishing on larval production is that while fishery scientists can sample the spatial distribution of individuals, the patterns of gamete dispersion have been observed in only a few cases (Pennington 1985; Wahle and Peckham 1999; Levitan 2002), and they would be difficult and expensive to quantify for each harvested population. Temporal variability in spatial distributions, owing to either density-dependence or seasonal behavioral aggregations for spawning, is also poorly known (Shepherd and Partington 1995; Levitan 2002). Similarly, spatial and temporal variability in flow conditions, turbulent interactions with substrate, and spatial variability in gamete properties across physical gradients (e.g., exposure, freshwater influence) all influence gamete production and dispersal at much smaller scales than that of either fishing or fishery management (Levitan 1991; Berger and Jelinski 2008). To address these issues, we apply an existing model of larval production using an assumed, centered unimodal distribution of dispersion distances, over a range of the uncertain dispersion distances (Lundquist and Botsford 2004). Presuming that actual dispersion is unimodal and centered on the origin, the results describe possible behavior over a range of dispersion distances. We apply this approach to the red sea urchin, *Strongylocentrotus franciscanus*, fishery in northern California, USA, as an example.

The red sea urchin fishery began in the 1970s in southern California, and expanded to northern California in the early 1980s. Landings peaked in northern California in 1988 at a level of 30 million pounds (1 lb = 0.4536 kg), and has drastically declined in the mid-1990s to the current level approximately 10% of the maximum harvest (Kalvass and Rogers-Bennett 2004). Continued intensive harvest has changed the size structure to that of a "recruitment" fishery, such that much of the catch is represented by red sea urchins that have only recently grown beyond the legal size limit of 89 mm (Kalvass and Rogers-Bennett 2004). Fishery managers observed a concurrent decline in the density of red sea urchins from an average of 1.7·m^{-2} in 1989 to 0.6·m^{-2} in 1994 (Kalvass and Hendrix 1997).

Red sea urchins are hypothesized to exhibit two different Allee effects. First, juvenile urchins reside beneath the spine canopy of the adults, receiving protection from predators and possibly gaining increased food supply (Tegner and Dayton 1977). Second, the Allee effect addressed here: reduced fertilization success at low density results from increased gamete dilution as distance between spawners is increased at lower densities (Levitan et al. 1991; 1992). Some aspects of this Allee effect in benthic marine invertebrates have been studied. Fertilization declines as density decreases (Levitan 1991; Yund 1995), as wave action increases (Pennington 1985; Denny and Shibata 1989), and as distance between spawners increases (Pennington 1985; Yund 1990; Levitan et al. 1992).

Fishery management models for the red sea urchin have included Allee effects in several ways. Botsford et al. (1993) chose a critical threshold of 0.7·m^{-2} of spawner density for fertilization success in their model of a rotating harvest scenario for this fished invertebrate. Pfister and Bradbury (1996) also modeled a rotating harvest strategy for red sea urchins, using two mathematical representations of an Allee effect: (*i*) a nonlinear Type II functional response when the population dropped below 50% of preharvest levels, and (*ii*) a more extreme linearly decreasing function with decreasing density. Quinn et al. (1993) used a log-linear relationship between fertilization success and average nearest neighbor distance based on Denny and Shibata (1989) to represent an Allee effect in *S. franciscanus* populations. Each of these models chose density-dependent relationships somewhat arbitrarily, based on limited field and laboratory studies of the fertilization Allee effect (Pennington 1985; Levitan et al. 1991, 1992). None of these models accounted for the effects of variability in density between sites or the effect of aggregation.

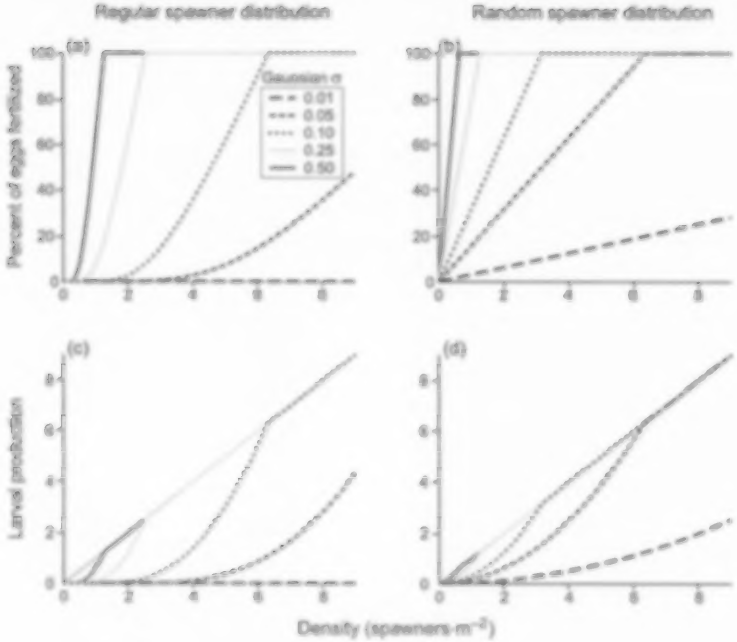
Here we use our fertilization model (Lundquist and Botsford 2004) to assess the implications of the observed distributions of adult spawners of the red sea urchin at various locations in northern California, i.e., to determine the larval production implied by these distributions. Because the mechanisms of fertilization are poorly known, we use a range of variability in the spatial scale of gamete dispersion to assess possible effects. Our specific goals are (*i*) to sample spatial variation in density and the degree of aggregation, (*ii*) to estimate larval production implied by these spatial distributions, and (*iii*) to compare current with pre-fishery density distributions and larval production.

Materials and methods

Sampling sites

Our sites were selected to include one sampling location approximately every 10 min of latitude over the range of

Fig. 1. Fertilization and larval production for Gaussian shaped gamete dispersal distributions, from Lundquist and Botsford (2004). Percentage of eggs fertilized versus density (*a* and *b*) and larval production versus density (*c* and *d*) for regularly spaced and randomly spaced distributions of spawners.



the northern California fishery between Bodega Bay (38°19.2′N) and Caspar (39°21.5′N) (Fig. 2). Red sea urchins are fished from four major ports in northern California (Bodega Bay, Point Arena, Albion, and Fort Bragg). The sites included two adjacent reserve and fished locations (Caspar and Bodega Bay). General habitat characteristics of most sites included a relatively exposed coastline, rocky substrate, and a surface kelp canopy of the bull kelp *Nereocystis leutkeana*. In most years, Bodega Bay lacks both a surface canopy of *N. luetkeana* and an extensive understory canopy of *Pterygophora californica*, although periodic accumulations of drift algae from northern sites do occur. The primary predators observed at our study sites are the sea stars *Pycnopodia helianthoides* and *Pisaster* spp. (C.J. Lundquist, personal observation). Sites exhibit some local heterogeneity of substrate, including kelp cover, substrate type (rock or sand), and landscape rugosity.

Field collection

Red sea urchin populations were surveyed using 5 m × 30 m transects. Over 7000 m^2 of sea urchin habitat was sampled, primarily in the summer of 1996, with additional collections in summer of 1997, and spring and fall of 1998. Transect lines were dropped from the surface to minimize bias in selecting areas to be sampled.

We compared methods of transect data collection to determine the survey method that minimized the time required per amount of area surveyed while estimating density at a fine enough spatial resolution to capture the effects of aggregation on reproductive dynamics for the fertilization model. We tested three different sampling procedures: (*i*) labor intensive mapping of individual sea urchin locations within the transect area, (*ii*) sea urchin counts within 0.5 m × 0.5 m quadrats (600 counts per transect), and (*iii*) sea urchin counts within 0.5 m × 1 m quadrats (300 counts per transect). Comparable simulations with the two quadrat sampling methods differed by less than 5% in average fertilization percentage in all but one case (Lundquist 2000). Mapped transect methods usually gave slightly higher values than both quadrat sampling methods, although the maximum difference in fertilization between mapping methods and quadrat sampling methods was less than 12%. We concluded that the sampling strategy of 0.5 m × 1 m quadrats, which required the least effort, was the most efficient method for describing the local spatial distribution.

For each 5 m × 30 m transect, SCUBA divers recorded the number of urchins within each 0.5 m × 1 m quadrat. Forty-two transects were completed in 1996 at 11 locations, 7 transects in 1997 at 2 locations, and 4 transects in 1998 at 3 locations. On nine occasions, logistics of subtidal sampling resulted in transects of dimensions of less than 5 m × 30 m. Urchins within and alongside the transects were collected and measured to determine the size frequency distribution and percentage of juveniles (0–60 mm), pre-legal adults (61–89 mm), and legal adults (>90 mm) at each site. The juvenile size category was chosen based on growth models to include pre-reproductive individuals (Morgan et al. 2000). Destructive substrate sampling was not performed; thus, the proportion of individuals under 15 mm was likely underestimated.

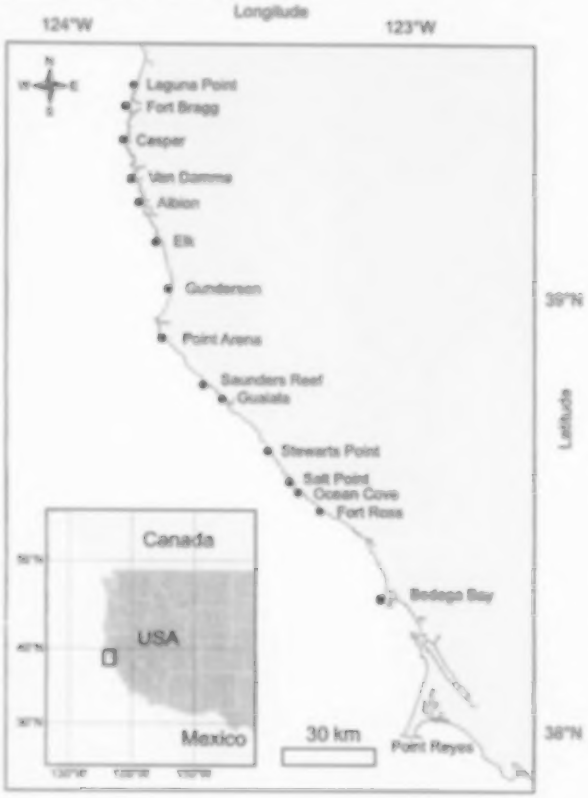


Fig. 2. Field sites in Sonoma and Mendocino counties, northern California. Headland features are located at Point Arena and Point Reyes, south of the Bodega Marine Reserve. California Department of Fish and Game (CDFG) surveys in 1988 and 1989 were located between Fort Ross and Laguna Point.

Data analysis

Average transect density was calculated as the total number of individuals divided by the total area sampled. Data were combined for 1996, 1997, and 1998 to calculate an average site density. Density estimates for each site were calculated from the mean of all quadrat counts from all transects completed at a site. Four of the 51 randomly dropped transects contained no sea urchins. As these areas were of similar habitat and topographic relief of transects that included sea urchins (often located within 100 m of a transect with sea urchins), these zero transects were included in calculations of density and fertilization.

We estimated the precision of our estimates by treating them as abundance estimates using standard quadrat sampling techniques (Seber 1982). These estimates were not straightforward because we did not know the total area of sea urchin habitat within each site. We therefore calculated the coefficient of variation, assuming our sample (total area of one transect = 300 quadrats = 150 m^2) was one of three proportions of the total area at a site: 1/10th, 1/20th, and 1/50th of the total area sampled.

To determine whether populations were aggregated, we used 0.5 m × 1 m quadrat counts to calculate Morisita's standardized index of dispersion (MSID):

$$\mathrm{MSID} = n\left[\frac{\Sigma x^2 - \Sigma x}{(\Sigma x)^2 - \Sigma x}\right]$$

where n represents the sample size, and x represents the quadrat count (Krebs 1989). The index is standardized for density and sample size, and ranges from −1.0 to +1.0. An index of 0.0 indicates a random pattern of spatial dispersion, positive indices indicate aggregated patterns, and negative indices indicate uniform or regular patterns of dispersion. Index values greater than 0.5 indicate that a transect is significantly more aggregated than a randomly distributed population. The average MSID at each site was calculated using all quadrat data from all transects at that site. MSIDs for the four transects with no urchins were undefined and were not included in the average for each site.

Fertilization model

To estimate the reproductive success implied by the observed densities of urchins at each site, we used the individually based simulation model of fertilization presented in Lundquist and Botsford (2004). For each distribution, we created a mapped spatial distribution of individual urchins based on observed transect data, and we estimated fertilization success based on theoretical distributions of egg fertilization rates with distance from male spawners. Empirical data (number of urchins per 0.5 m × 1 m quadrat) were converted to mapped spatial distributions by generating uniform random x and y coordinates to place each sea urchin within each quadrat. The gender of each sea urchin was determined randomly, assuming a 1:1 sex ratio. We used a uniform random number generator to determine whether a sea urchin was a reproductive adult (>60 mm) based on the percentage of adults found in size frequency distributions at each site (Morgan et al. 2000).

We simulated four different distributions of gamete dispersion to graphically represent a range of possible gamete dispersion patterns for the red sea urchin in northern California. These dispersal distributions were originally developed from empirical fertilization data based on a congener *S. droebachiensis* in a unidirectional tidal current (Pennington 1985; Lundquist 2000). Further empirical studies have shown similar functional forms of the relationship of fertilization success with distance, including observations with additional complexities due to aggregation and spawner density, in both unidirectional and oscillatory flow conditions for multiple sea urchin species, including *S. franciscanus* (Levitan et al. 1992; Wahle and Peckham 1999; Levitan 2002). We used both a relatively narrow distribution and a relatively broad distribution, with and without an extended tail, to represent the range of responses shown in Fig. 1. The extended tail represented successful fertilization at distances beyond that which fertilization models of gamete kinetics based on diffusion of gametes would predict (Denny and Shibata 1989). There is empirical evidence that fertilization at these distances occurs (Babcock and Mundy 1992; Thomas 1994). The four examples are identified as narrow with a tail (NT), narrow with no tail (NNT), broad with a tail (BT), and broad with no tail (BNT) (Table 1). The gam-

Table 1. Gamete dispersal distributions.

Distribution	Description	Equation
NT	Narrow with a tail	$y = e^{-5\sqrt{x}}$ for 0<x<0.18; y = 0.05 for 0.18<x<5.0
NNT	Narrow with no tail	$y = e^{-5\sqrt{x}}$
ET	Broad with a tail	$y = e^{-1.5x}$ for 0<x<1.5; y = 0.10 for 1.5<x<5.0
BNT	Broad with no tail	$y = e^{-1.5x}$

Note: y represents percent of eggs fertilized and x represents density of spawners·m^{-2}.

ete dispersal distributions with tails were normalized to the corresponding distributions without tails to maintain a constant sperm supply.

To estimate the variability in fertilization at each site, we performed 100 simulations for each transect for each of the four gamete dispersion distributions, varying the random placement and gender of spawners within each quadrat. The variability in these simulations represented both uncertainty in measurement, as we did not know the exact location of sea urchins within each quadrat, and interannual variability in sea urchin distributions as sea urchins likely reform spatial distributions during each spawning season. The percentage of eggs fertilized for each adult female was calculated as the cumulative percentage of sperm reaching each adult female from all adult males within the grid, based on the sperm dispersal distribution. Average fertilization was calculated for each simulation as the average fertilization percentage of all females in a given simulation.

To test whether aggregation of adults in observed distributions increases fertilization success, we compared the fertilization results based on observed spatial distributions with a simulation using the same density as observed transects, but with sea urchin locations within the transect generated from a uniform random distribution.

Larval production

Larval production for each transect was calculated as the adult density (sea urchins > 60 mm) × a polynomial fit to fertilization success versus adult density for both observed and random simulations. As we were interested in the proportional decrease in fertilized larvae rather than estimating the exact numbers of gametes released and fertilized, this calculation of larval production allowed us to compare declines in larval production relative to declines in adult density. To assess the Allee effect on current larval production, the maximum possible total larval production was calculated for the transects from 1996–1998 as the total larval production assuming no Allee effect (i.e., 100% fertilization success).

We also obtained data from the California Department of Fish and Game (CDFG) (P. Kalvass, Department of Fish and Game, personal communication 1999) from transects sampled at 14 locations between Fort Ross, Sonoma County, and Laguna Point in Mendocino County (Fig. 2) in 1988 (n = 105) and 1989 (n = 76). We used CDFG data from 1988–1989 as a proxy for pre-harvest conditions, as these were the first years that sea urchin data were collected in northern California. CDFG transects sampled the number of sea urchins per 1 m × 5 m quadrat along a 30 m transect line. We used size frequencies collected at Point Cabrillo Marine Reserve in Mendocino County (Fig. 2) in 1989 (Morgan 1997) to estimate the percentage of adults to compute adult density for the CDFG transects.

We compared harvested (1996–1998) and pre-harvest (1988–1989) estimates of larval production. As with the transects from 1996 to 1998, to estimate total larval production from the CDFG data, we calculated larval production for each CDFG transect from 1988 to 1989 using the estimated relationship between larval production and adult density generated from observed transect simulations. Total larval production for the transects from 1988 to 1989 was calculated as the sum of larval production for all individual transects, normalized to the total area sampled. We used the decline in total larval production since the inception of the fishery, concurrent with changes in density and aggregation, to determine the relative contribution of declines in density since inception of the fishery, the fertilization Allee effect, and the effect of aggregation. The fertilization Allee effect was estimated by comparing larval production from simulation models assuming no Allee effect (i.e., 100% fertilization success) with those with the four simulated gamete dispersal curves. The effect of aggregation was estimated by comparing larval production from simulation models of aggregated individual distributions with observed quadrats compared with random distributions within entire transects.

Results

Density

Average density in 1996–1998 varied with site between 0.1 sea urchins·m^{-2} and 1.6 sea urchins·m^{-2} (Fig. 3*a*; Table 2), with no discernable pattern over space. Some sites (Albion, Saunders) had much higher average densities than neighboring sites (Fig. 3*a*). Juvenile density was higher at some sites north of Point Arena (Elk, Albion, and Caspar Reserve), with all other sites having low densities of juveniles in 1996.

Reserves had higher average densities than adjacent fished sites (reserves, 1.1·m^{-2}; fished site, 0.8·m^{-2}) (Figs. 3*a* and 3*b*). However, owing to the high variance in density between transects at both fished and reserve locations, this difference was not significant (t = –0.901, p = 0.19). Both reserve locations showed higher densities of legal-sized adults than adjacent fished sites. One location, Caspar Reserve, had much higher numbers of juveniles compared to its adjacent fished site (Fig. 3*a*).

Coefficients of variation for most sites indicated between 5% and 15% error in estimates of total abundance, based on the quadrat count estimates (Table 2). Larger percentage error in estimation of abundance occurred for sites with lower sampling effort. Some sites showed high variability in sea urchin density, which in turn increased the variance of the stratified estimate of abundance.

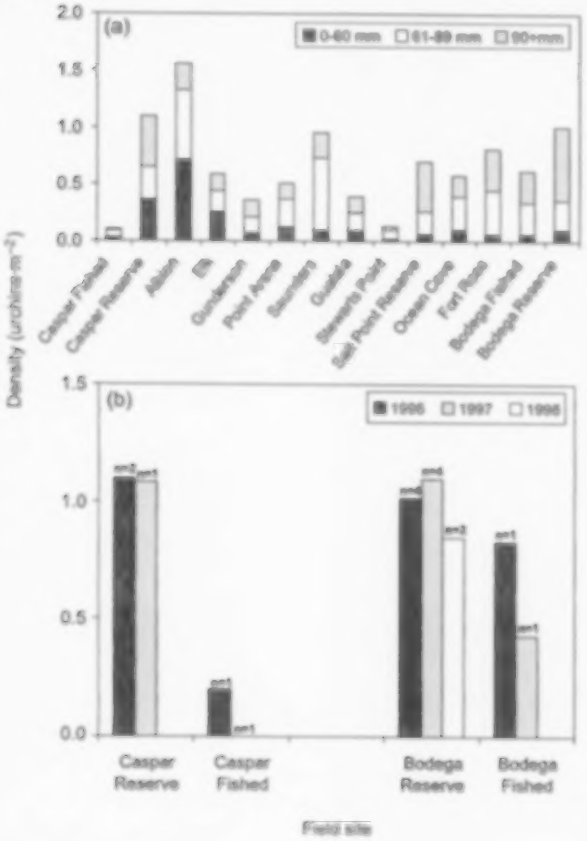


Fig. 3. (*a*) Observed red sea urchin densities from transect data for field sites in northern California. Densities are separated into juveniles (0–60 mm), pre-legal adults (61–89 mm), and legal adults (90+ mm); (*b*) Density at adjacent fished and reserve locations in 1996–1998 (n = number of transects of 300 of 0.5 m × 1 m quadrats).

Aggregation

All transects exhibited significant aggregation using the Morisita's standardized index of dispersion (MSID) (Fig. 4). Average MSID indices for each site showed no discernable pattern across space (Fig. 4*a*), but areas of low density (less than 0.5·m^{-2}) had higher levels of aggregation (Fig. 4*b*). Aggregation (MSID) was significantly negatively correlated with both adult density (Spearman rank correlation coefficient $r_S = -0.713$, $p < 0.0001$) and total density ($r_S = -0.709$, $p < 0.0001$).

Larval production

Larval production showed similar patterns across sites to those of sea urchin density (Figs. 5*a*–5*d*). Correlations between adult density and average larval production at a site were significantly positive for all four gamete dispersal distributions (BT: $r = 0.810$, $p < 0.01$; BNT: $r = 0.825$, $p < 0.005$; NT: $r = 0.828$, $p < 0.005$; NNT: $r = 0.712$, $p < 0.05$). While low density sites may have high values of fertilization success due to high levels of aggregation (i.e., Stewarts Point), the few individuals and thus small number of fertile gametes produced relative to high density sites implies that these low density sites are less likely source populations for larval export.

To determine the effect of density and fertilization on the stock–recruitment relationship for red sea urchins in northern California, we examined the relationship between adult density (stock) and larval production (recruitment). For the broad gamete distribution simulations, plots of larval production versus adult density indicated only a slight deviation from the linear relationship expected if there was no effect of fertilization on larval production at densities above 0.5·m^{-2} (Figs. 6*a* and 6*b*). At lower densities (<0.5·m^{-2}), the relationship between larval production and adult density demonstrates a gradual nonlinear Allee effect. As noted in Lundquist and Botsford (2004), this Allee effect does not appear in the form of a strict decrease to zero at a threshold, but rather as a slight nonlinear decrease in larval production as density decreases. Larval production for narrow gamete distribution simulations showed reduced larval production at all densities and an approximately linear relationship between larval production and adult density (Figs. 6*c* and 6*d*).

Larval production from observed transect data showed higher values of larval production compared to the randomly generated simulations (Figs. 6*a*–6*d*). The random simulations exhibited a more pronounced decrease in larval production at low density, implying that aggregation reduces the influence of Allee effects at low density.

Distribution of estimated larval production across all northern California sites for the four gamete dispersal simulations showed similar distributions of larval production for Allee effect simulations of broad gamete distributions with aggregated distributions compared to simulations with no Allee effect (Fig. 7). Median values of simulations with random spatial distributions were shifted to smaller estimates of larval production compared to simulations with aggregated distributions. Distributions of larval production were shifted substantially toward smaller values for simulations with narrow gamete distributions. Tailed distributions increased in maximum value from 0.779 for NNT to 1.638 for NT simulations; the relative increases between the BNT and BT distribution were minimal (Fig. 7).

Comparisons with pre-harvest years

Estimates of density calculated for 1988 (n = 105) and 1989 (n = 76) CDFG transect data showed a wider range of densities than is present in data collected in 1996–1998 for both fished and reserve locations (Fig. 8*a*–8*c*). Less than half of all 1988–1989 transects had total densities of less than 0.5·m^{-2}, with some transects including densities of up to 20·m^{-2} (Fig. 8*a*). In contrast, half of the 1996–1998 fished transect data (n = 40) had density values less than 0.5·m^{-2} and the distribution of density values ranged between 0 and 5·m^{-2} (Fig. 8*b*). Reserve transects in 1996–1998 had a more uniform distribution than the fished transect data, with less than half of the transects at densities less than 0.5·m^{-2} (Fig. 8*c*), although our analysis is limited, owing to the small sample size (n = 13).

To determine the relative effect of the fishery on larval production, we estimated total larval production for 1988–1989 by using the regression relationships of larval production with density for the four gamete dispersal distributions

Table 2. Average red sea urchin density and aggregation at northern California sites. Aggregation indices and coefficients of variation as explained in text. CV1, CV2, and CV3 refer to coefficient of variation based on assumption of sampling of 1/10th, 1/20th, and 1/50th of the total habitat, or total area = 1500 m^2, 3000 m^2, or 7500 m^2, respectively.

Site	No. transects	Area (m^2)	No. urchins	Mean ($\cdot m^{-2}$)	SD	Variance/ mean	MSID[a]	CV1	CV2	CV3
Caspar fished	2	300	30	0.100	0.962	9.250	0.5592	35.12	37.25	38.47
Caspar reserve	3	450	491	1.091	2.461	5.551	0.5031	6.29	6.93	7.29
Albion	5	675	1051	1.557	3.455	7.665	0.5017	4.48	5.32	5.76
Elk	4	550	327	0.595	2.965	14.778	0.5113	11.97	13.59	14.48
Gunderson	4	600	218	0.363	2.044	11.510	0.5132	12.58	14.53	15.58
Point Arena	6	825	424	0.514	2.098	8.566	0.5051	6.74	8.56	9.48
Saunders	2	300	286	0.953	2.399	6.038	0.5053	9.19	9.74	10.06
Gualala	2	300	118	0.393	1.928	9.461	0.5200	17.90	18.99	19.61
Stewarts	1	150	19	0.127	1.297	13.250	0.6536	56.12	57.66	58.56
Salt Point	7	837.5	587	0.701	2.541	9.208	0.5038	5.89	7.52	8.35
Ocean Cove	4	525	305	0.581	2.598	11.620	0.5095	11.13	12.54	13.31
Fort Ross	1	150	122	0.813	2.017	5.005	0.5055	13.58	13.96	14.17
Bodega fished	2	300	188	0.627	1.587	4.016	0.5053	10.38	11.01	11.37
Bodega reserve	10	1275	1277	1.001	2.602	6.763	0.5013	1.99	3.90	4.69

Note: All variance:mean ratios were significant at $p < 0.01$. All Morisita indices of dispersion (MSID) were significant at $p < 0.05$.
[a]MSID (aggregation) calculations do not include zero transects.

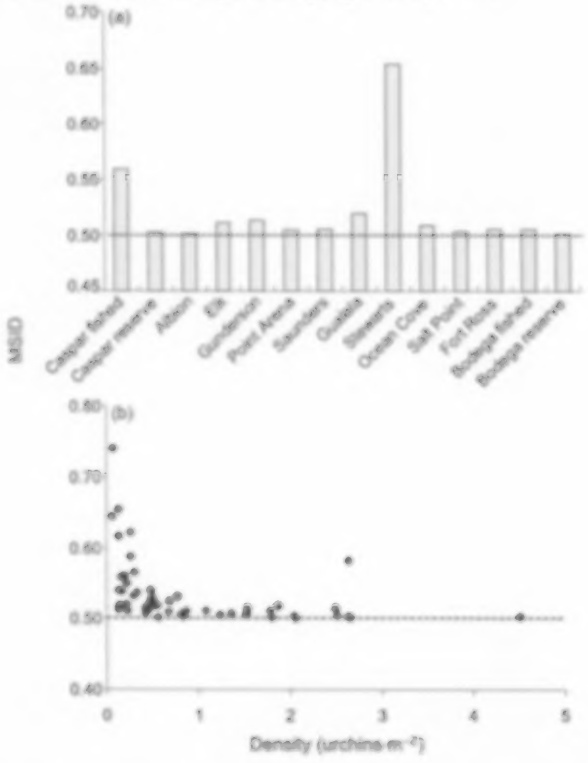


Fig. 4. (*a*) Standardized Morisita index of dispersion (MSID) per site, from north to south; (*b*) MSID versus sea urchin density·m^{-2}. Broken line represents significant aggregation at $p < 0.05$.

from the observed transect data for the period 1996–1998. For transects with densities of less than 0.5 urchins·m^{-2}, we used a best fit polynomial regression to account for nonlinear decreases in larval production at low densities (BT: $y = -0.0517x^3 + 0.1992x^2 + 0.8175x$, $R^2 = 0.9990$; BNT: $y = -0.0539x^3 + 0.2386x^2 + 0.7256x$, $R^2 = 0.9977$; NT: $y = -0.1579x^3 + 0.5907x^2 - 0.0551x$, $R^2 = 0.8833$; NNT: $y = 0.0276x^3 - 0.0320x^2 + 0.1645x$, $R^2 = 0.8787$). For transects with densities greater than 0.5·m^{-2} we used a linear regression through the origin to determine relative larval production (BT: $y = 0.9867x$, $R^2 = 0.9974$; BNT: $y = 0.9586x$, $R^2 = 0.9933$; NT: $= 0.4306x$, $R^2 = 0.8370$; NNT: $y = 0.2093x$, $R^2 = 0.8347$).

Total larval production for fished transects in 1996–1998 ($n = 40$) was approximately one-third of the estimated 1988–1989 larval production values for the four simulated gamete dispersal distributions (Fig. 9*a*). The decrease in larval production would have been between 3% and 23% more severe, relative to 1988–1989 CDFG values, if sea urchins had been distributed randomly (Fig. 9*a*). For 1996–1998 fished transects, the estimates based on random transects represent decreases in larval production of 9%–69% compared with observed transects. The NNT simulations had noticeably larger decreases owing to the limited distance of effective fertilization of this gamete dispersal distribution. Larval production for 1996–1998 reserve transects ($n = 13$) was approximately 50% of 1988–1989 estimated larval production, with similar relative declines with random transects, although decreases were less severe in NNT simulations, owing to higher adult densities at reserve sites (Fig. 9*a*).

Total decreases in larval production in 1988–1989 relative to maximum possible larval production in 1988–1989 (assuming 100% fertilization success) ranged from less than 10% for the two broad gamete distribution simulations (BT and BNT) to 58% and 79% for the NT and NNT simulations (Fig. 9*b*). Observed 1996–1998 estimates for fished transects showed similar decreases relative to maximum possible larval production in 1996–1998 with broad gamete distribution (BT and BNT) simulations producing mild decreases in larval production of less than 10%, and NT and NNT simulations producing severe decreases of 60%–80% (Fig. 9*b*).

Fig. 5. Larval production (adult density times average fertilization percentage) per site (north to south) for (*a*) a broad sperm dispersal distribution with an extended tail (BT); (*b*) a broad sperm dispersal distribution with no tail (BNT); (*c*) a narrow sperm dispersal distribution with an extended tail (NT); and (*d*) a narrow sperm dispersal distribution with no tail (NNT).

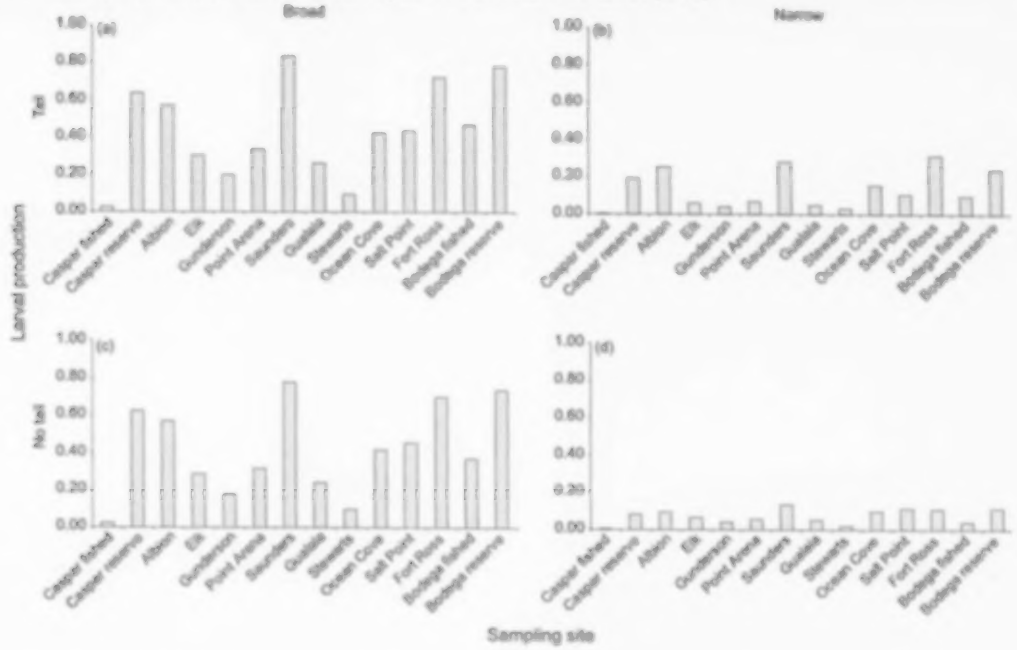
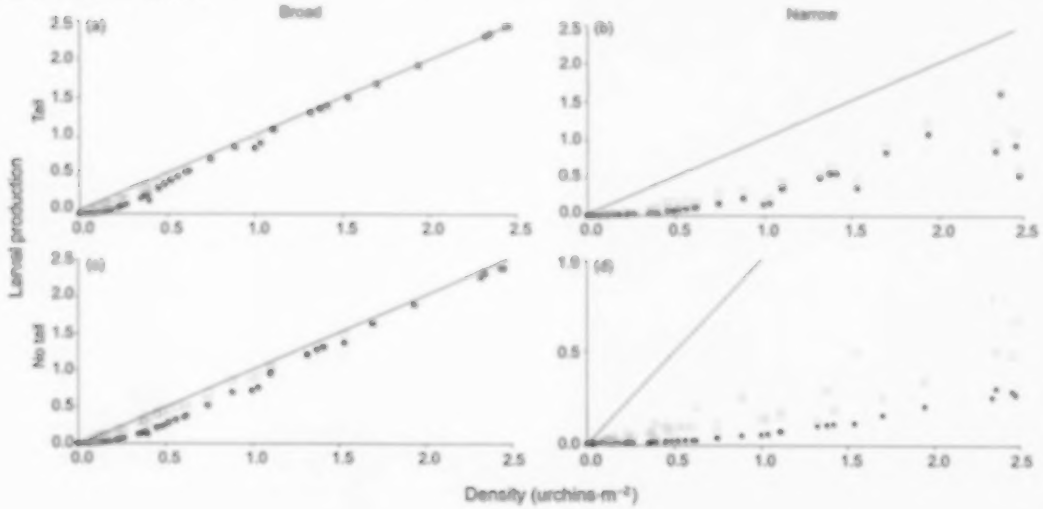
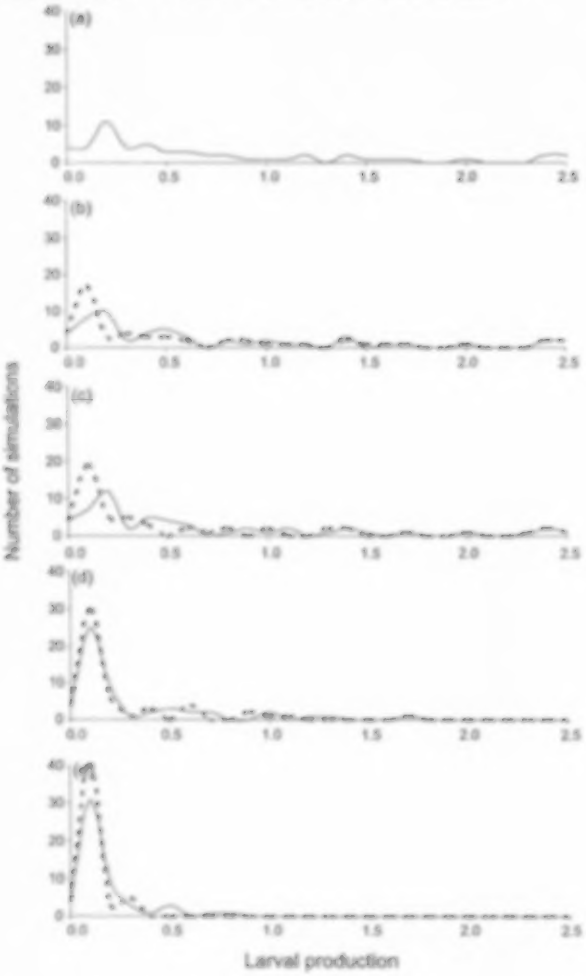


Fig. 6. Larval production (adult density × average fertilization percentage) versus adult density for simulation runs with observed transect data (filled squares) and with randomly generated spatial distributions (open squares) for (*a*) a broad sperm dispersal distribution with an extended tail (BT); (*b*) a broad sperm dispersal distribution with no tail (BNT); (*c*) a narrow sperm dispersal distribution with an extended tail (NT); and (*d*) a narrow sperm dispersal distribution with no tail (NNT). A 45° line is presented to show expected larval production given 100% fertilization success.



We estimated the decrease in larval production expected due to density alone, comparing average adult sea urchin density from 1988–1989 CDFG transects (1.415·m^{-2}) versus average density from 1996–1998 fished transects (0.611·m^{-2}). Average adult density declined to 43.2% of the 1988–1989 value, compared with a mean decrease in larval production across all four gamete dispersal distributions to 33.8% of 1988–1989 larval production based on 1996–1998 observed transects (Fig. 9*a*). Averaging across the four gamete dispersal distributions, approximately 21.7 ± 4.1% of the

Fig. 7. Distribution of estimated larval production across all northern California sites for simulations with (*a*) no Allee effect (e.g., 100% fertilization success); (*b*) a broad sperm dispersal distribution with an extended tail (BT); (*c*) a broad sperm dispersal distribution with no tail (BNT); (*d*) a narrow sperm dispersal distribution with an extended tail (NT); and (*e*) a narrow sperm dispersal distribution with no tail (NNT). Unbroken lines denote aggregated spatial distributions; broken lines denote random spatial distributions.

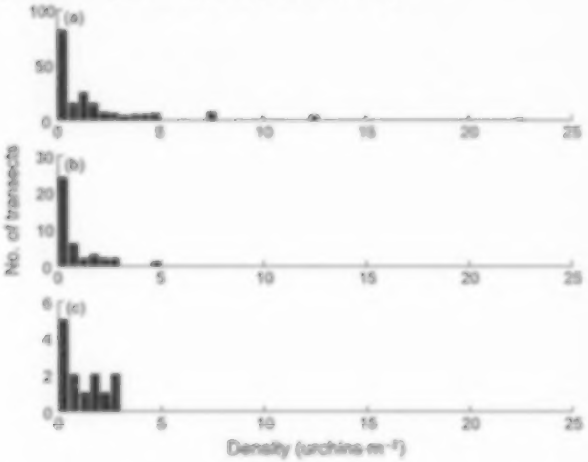


realized decrease in larval production can be explained by the fertilization Allee effect. This decrease would be 45.2 ± 21.7% (23.5% more severe) if sea urchins were distributed randomly rather than as observed in spatial aggregations. The effect of aggregation is most apparent in the narrowly distributed gamete dispersal distributions.

Discussion

Our results for the northern California red sea urchin fishery demonstrate that fishing can decrease the larval production of a broadcast spawning population as decreased fertilization efficiency at low density results in low levels of larval production. Mean density in 1996–1998 was

Fig. 8. Distribution of density values for (*a*) CDFG 1988–1989 transect data (n = 181 transects measuring 30 m^2 and consisting of six 1 m × 5 m quadrats); (*b*) transect data for this study (1996–1998) at fished locations (n = 40 transects, each comprising 300 quadrats measuring 0.5 m × 1 m); and (*c*) transect data for this study (1996–1998) at reserve locations (n = 13 transects each comprising 300 quadrats measuring 0.5 m × 1 m).

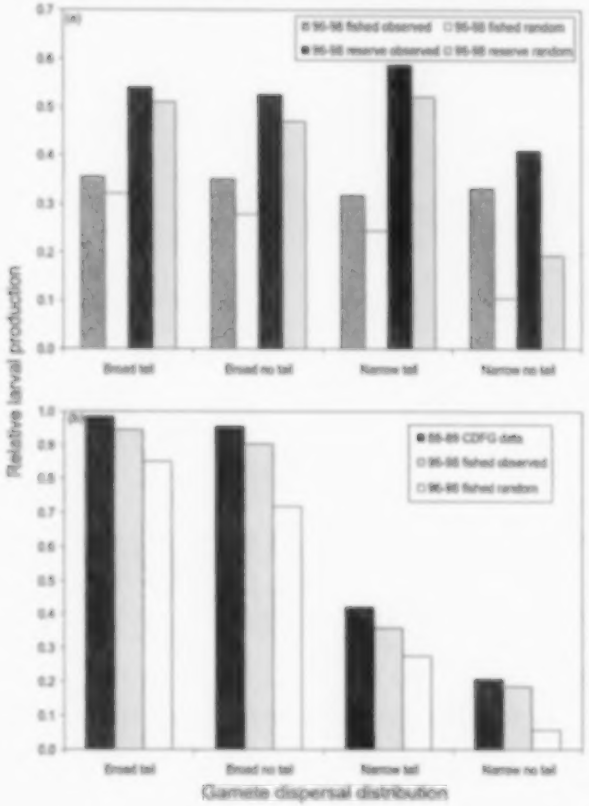


43.2% of the calculated value in 1988–1989, while mean larval production was estimated from the model as 33.8% of the 1988–1989 value. The additional decrease in larval production (21.7%), beyond that attributed to declines in density, can be attributed to the fertilization Allee effect. Aggregating behavior also shows a significant role in reducing the potentially more severe Allee fertilization effect, with an additional decrease of 23.5% if sea urchins were distributed randomly.

The relative decrease in larval production owing to the Allee fertilization effect will be more severe if average transect density (average 1996–1998 adult density = 0.611·m^{-2}) decreases below levels of 0.5·m^{-2}, a level at which decreases in recruitment owing to the fertilization Allee effect are particularly severe. Our model implies that roughly half of the individuals in the population are currently experiencing density levels sufficient for fertilization success. While large proportions of low-density observations occur in all years, the primary difference between 1988–1989 and 1996–1998 lies in the lack of high density (>5·m^{-2}) transects in 1996–1998.

The Allee effect is also smaller than might be predicted, owing to a lack of the restrictive theoretical conditions that result in a decrease to zero below a threshold density, as assumed in many fishery models that incorporate Allee effects (Botsford et al. 1993; Quinn et al. 1993; Pfister and Bradbury 1996). An analytical model of fertilization has shown that strict threshold decreases occur only if spawners are located in a regular equidistant pattern with a sharply declining sperm dispersal distribution (Lundquist and Botsford 2004). A gradually decaying sperm dispersal distribution (as is empirically suggested for broadcast spawning) or a random distribution of individuals both change the dynamics and lessen the effect of this critical threshold. This implies that it is the stochastic nature of the spatial distribution of

Fig. 9. Comparison of larval production estimated for 1988–1989 (88–89) California Department of Fish and Game (CDFG) data and for 1996–1998 (96–98) transect data for the four sperm dispersal distributions. (*a*) Larval production relative to 1988–1989 larval production for observed and random simulations of 1996–1998 fished and reserve transects; (*b*) larval production relative to maximum possible larval production (i.e., assuming no Allee effect and 100% fertilization success) for 1988–1989 CDFG data, and observed and random simulations of 1996–1998 fished transects.



individuals that is responsible for the lack of strict threshold Allee effect.

While we illustrate our simulations based on fertilization dynamics that are similar in functional form and magnitude of fertilization success with distance to numerous field studies on multiple sea urchin species including *S. franciscanus* (Pennington 1985; Levitan et al. 1992; Wahle and Peckham 1999), actual conditions in northern California during red sea urchin spawning are unknown. While some empirical observations of broadcast spawning have been during low-flow conditions (Breen and Adkins 1980; Stekoll and Shirley 1993), other species have been observed spawning during turbulent conditions (Desrosiers and Dube 1993; Serrao et al. 1996). The spawning period of red sea urchins is predicted to occur during the upwelling season in northern California, implying that the sea urchins are spawning during high-flow conditions (Bennett and Giese 1955; Pearse 1980). In that case, our results indicate that aggregation is particularly important for minimizing Allee effects by decreasing the distance between spawners. Further complexities influencing hydrodynamics at the scale of the individual sea urchin (e.g., turbulent interactions between flow and rocks, crevices, or channels; oscillatory flows and other complex flow dynamics) will undoubtedly result in more complex expression of the gamete dispersal distribution that we did not evaluate here (Denny and Shibata 1989; Denny et al. 1992; Gaylord et al. 2002). Regardless, the four gamete dispersal distributions that we simulated cover four widely varying dispersal curves, with fertilization dynamics at common densities of *S. franciscanus* ranging from Gaussian to linear to highly skewed.

The lack of inclusion of sea urchin size (and thus gonad volume) as an additional factor affecting fertilization in our models to determine larval production implies that our model gives a conservative underestimate of the actual decrease in larval production due to fishing, as fishing has truncated the size distribution of sea urchins. Similarly, egg size, sperm velocity, sperm longevity, and gamete volume differ with sea urchin size, across habitat gradients, and between strongylocentroid species, with varying effects on fertilization dynamics (Levitan 1991; 2002; Berger and Jelinski 2008). Other models of fertilization Allee effects that include gonad volume have predicted much larger decreases in egg production, as density of spawners decrease (Claereboudt 1999; Meidel and Scheibling 2001). Field experiments have also shown that sea urchins at higher densities often have lower gonad volumes: a complexity that could reduce the influence of the fertilization Allee effect if larger gonads compensate for reduced adult density (Levitan 2002). The assumption of synchronous spawning in our model is also conservative, as sea urchins (and other benthic invertebrates) in nature are rarely observed to spawn synchronously (Pennington 1985; Levitan 1988; Levitan and Sewell 1998). Violation of this assumption would result in further decreases in the overall scale of larval production, rather than interacting with the dynamics modeled here. There is also little evidence for increased aggregative behavior during spawning for red sea urchins (Pennington 1985; Levitan 1988; Pearse et al. 1988). However, we caution that the lack of regular observations of aggregative behavior for spawning and synchronous spawning events should not be used to infer that these behaviors do not occur, as spawning events (and associated aggregative behaviors) are rarely observed in situ.

A major concern in the "fishing down" of this sea urchin population is that denser patches of benthic invertebrates are serially depleted, with catch and CPUE remaining relatively high until these dense populations no longer remain (Orensanz et al. 1998; Karpov et al. 2000). The range of density has clearly decreased since the CDFG surveys during 1988–1989, as no sites were observed with densities greater than 5.0·m^{-2}. The highest density sites (e.g., Albion) were composed primarily of juveniles, and the fishery catches have shown higher dependence on urchins that have just recently entered the legal size category (Kalvass and Rogers-Bennett 2004). A large proportion of the larval production during 1996–1998 could be attributed to the adult densities present in the five densest sites, while the five densest sites in 1988–1989 accounted for only 18% of the larval production. This concentration of larval production in fewer locations than in earlier years should cause concern, as the fishery is

relying on fewer areas to provide the majority of larvae to the population. In addition, the average density of the five most dense transects has decreased from approximately 12·m^{-2} in 1988–1989 to approximately 2·m^{-2} in 1996–1998, a 6-fold decrease in density for these highest density areas. As larval dispersal of sea urchins in the region is estimated to be on the scale of tens of kilometres (Morgan et al. 2000), this reduction in spawner density and associated larval production from key source locations will have relevance to fishery performance across the broader coastline.

We use larval supply as an estimate of recruitment, and do not address the complex relationship between larval supply and juvenile settlement. Erratic recruitment of sea urchins in northern California has been noted by many researchers (Ebert et al. 1994; Lundquist et al. 2000). However, the exact coupling between fertilized eggs through planktonic larval stages, settlement to the benthos and survival to reproductive adults is not known. Turbulent flows affect the fertilization processes addressed here, as well as influencing other biological processes such as settlement rates through advection and transport of larvae (Butman 1987; Denny and Shibata 1989). We exclude an additional Allee effect from our model, that of protection of juvenile red sea urchins beneath adult spine canopies (Tegner and Dayton 1977). Inclusion of this Allee effect would be more important in a size-specific model, while within our model dynamics, it would show similar functional relationships to fecundity versus density.

Our average site estimates of density may have been affected by sampling error or insufficient sampling effort. Red sea urchins exhibit a patchy distribution, owing to their aggregative behavior as well as their tendency to reside in patchily distributed rocky reef habitat. Variation in density and other population parameters often occurs over smaller spatial scales than that over which populations are managed (McShane 1994; McShane et al. 1994). As such, our limited sampling effort was more efficient in describing the range of densities within the entire northern California population rather than density within individual sites, as shown by the high variance in density among transects within a site.

In summary, we determined that the fishery has caused a reduction in larval production. However, the spatial variation in density, both within and between sites, was large, and the influence of local densities is greater than the influence of the Allee effect on this decrease in larval production. In recent years, the fishery has modified the local density distribution, as there are now more extremely low density sites subject to Allee effects, and no high-density sites. The result is a decrease in larval production due to the fishery to levels of approximately one-third of pre-harvest larval production. A continuing trend of fishing out high density areas will only increase the likelihood of reproductive failure as average density of all areas approaches levels where fertilization is severely reduced due to Allee effects. Maintenance of broodstock in some areas protected as no-take reserves may be a means of increasing recruitment.

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Effects of spawning distribution on juvenile Atlantic salmon (*Salmo salar*) density and growth

Maxim A.K. Teichert, Anders Foldvik, Torbjørn Forseth, Ola Ugedal, Sigurd Einum, Anders G. Finstad, Richard D. Hedger, and Edwige Bellier

Abstract: We test whether the spatial distribution of spawning sites in a natural population is related to the density and individual growth rate of juvenile stream-dwelling salmonids. For this purpose, fine-scale data on the spatial distribution of Atlantic salmon (*Salmo salar*) nest sites, juvenile densities and body size, and habitat characteristics were combined. The area of nests summed over reaches of 25 m in length was positively correlated with local young-of-the-year (YOY) densities and was the strongest predictor of local densities. In turn, increased YOY densities were related to slower growth, and YOY density was the strongest predictor of YOY size. Measured habitat variables had little effect on YOY density over the spatial scale examined, which suggests that redistribution of YOY salmon according to habitat preferences was limited. The distribution of spawning sites was therefore the best predictor of local densities and subsequently YOY growth. Thus, during the YOY stage when dispersal is limited, patchy spawning distributions combined with local density-dependence may be the dominant factor responsible for spatial variation in growth.

Résumé : Nous vérifions si la répartition spatiale des sites de fraie dans une population naturelle est reliée à la densité et au taux de croissance individuelle des jeunes salmonidés d'eau courante. Pour ce faire, nous avons combiné des données à échelle fine sur la répartition spatiale des sites de nidification de saumons atlantiques (*Salmo salar*), sur les densités et les tailles corporelles des jeunes et sur les caractéristiques de l'habitat. Il existe une corrélation positive entre la surface cumulée des nids sur des sections de 25 m de longueur et la densité locale des jeunes de l'année (YOY); c'est la meilleure variable prédictive des densités locales. Par ailleurs, les densités accrues de YOY sont reliées à une croissance ralentie et la densité des YOY est la meilleure variable prédictive de la taille des YOY. Les variables mesurées de l'habitat ont peu d'effet sur la densité des YOY à l'échelle spatiale considérée, ce qui laisse croire qu'il y a une redistribution limitée des saumons YOY d'après leurs préférences d'habitat. La répartition des sites de fraie est donc la meilleure variable prédictive des densités locales et subséquemment de la croissance des YOY. Ainsi, durant le stade YOY pendant lequel la dispersion est limitée, la répartition contagieuse de la fraie, combinée à une densité-dépendance locale, peut être le principal facteur responsable de la variation spatiale de la croissance.

[Traduit par la Rédaction]

Introduction

In addition to the traditional view that juvenile salmonid performance in rivers is mainly determined by habitat conditions (Bovee 1986), there is now ample evidence that freshwater growth in stream-dwelling salmonids is strongly affected by fish density (Jenkins et al. 1999; Grant and Imre 2005; Einum et al. 2006). Local densities of juvenile Atlantic salmon (*Salmo salar*) may be strongly influenced by their habitat selection (Armstrong et al. 2003; Armstrong 2005; Hedger et al. 2005), in turn influencing performance through the resulting density differences (Harvey et al. 2005; Teichert et al. 2010). However, local densities may also be influenced by adult female habitat selection and the resulting spatial distribution of spawning sites within the river, and this is predicted to influence their population dynamics (Einum et al. 2008*a*).

The idea that spawning site distribution (i.e., the distribution of sites actually used for spawning in the relevant year, not all sites potentially suitable for spawning) may translate into juvenile growth rates via density dependence was first suggested by Crisp (1995) and has been inferred as being an important mechanism in density regulation (Armstrong 2005). Recent experimental work has provided empirical support for such links between the collective spatial decisions made by breeding adults and population consequences for offspring performance. By manipulating the spatial distribution, and hence local densities of eggs and juveniles in

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M.A. Teichert,[1] A. Foldvik, T. Forseth, O. Ugedal, A.G. Finstad, R.D. Hedger, and E. Bellier. Norwegian Institute for Nature Research, Tungasletta 2, N-7485 Trondheim, Norway.
S. Einum. Norwegian Institute for Nature Research, Tungasletta 2, N-7485 Trondheim, Norway; Norwegian University of Science and Technology, Centre for Conservation Biology, Department of Biology, Realfagbygget, NO-7491 Trondheim, Norway.

[1]Corresponding author (e-mail: maxim.teichert@nina.no).

stream sections, it has been shown that density effects (reduced growth and (or) survival at high density) operate at spatial scales far smaller than the scale of the population (Einum and Nislow 2005; Einum et al. 2006; Einum et al. 2008*b*). This pattern is likely due to the limited dispersal abilities of young-of-the-year (YOY) Atlantic salmon (Beall et al. 1994; Webb et al. 2001; Foldvik et al. 2010), where the majority of the offspring may remain within 20 m of the nest (Crisp 1995). Energetic costs of dispersal and (or) increased exposure to predators during dispersal are likely explanations for this phenomenon (Einum and Nislow 2005).

Whereas experimental studies provide support for a link between the distribution of spawning sites and resulting density-dependent juvenile performance, its importance for natural populations has remained largely unknown. Traditionally, studies dealing with the causes and consequences of density dependence in natural populations have been limited by the coarse spatial scales at which they are conducted (Einum and Nislow 2005). Hence, we designed a field study at a fine spatial scale, covering most of the recruitment area of a natural salmon population. Thus, we tested for (*i*) relationships between spawning distribution, habitat characteristics, and the resulting YOY density distribution and (*ii*) relationships between YOY density, habitat factors, and growth.

Materials and methods

Study area

Fieldwork was conducted in the River Børsa, central Norway (63°19′N, 10°40′E). The River Børsa is regulated for hydropower production with median and minimum summer water discharges of ~3 $m^3 \cdot s^{-1}$ and ~0.25 $m^3 \cdot s^{-1}$, an average width of 9.7 m, and a drainage area of 110 km^2. Anadromous salmonids are able to access this river to an upstream distance of 5.4 km. The lower 1.6 km of the river, starting about 700 m up from the estuary were recently (2003–2006) strongly modified, therefore only data from the uppermost 3.1 km of the river were used in our study. Atlantic salmon is the dominant species in the river, but smaller numbers of brown trout (*Salmo trutta*), three-spined stickleback (*Gasterosteus aculeatus*), European flounder (*Platichthys flesus*), and European eel (*Anguilla anguilla*) are also present (Johnsen and Hvidsten 2007).

Spawning survey

Nest distribution was mapped by walking the entire stretch accessible to anadromous salmonids and assigning global positioning system (GPS) references (average accuracy ±7 m) to individual nests or clusters of nests, during early December 2006, when all spawning activity had finished. The area of each individual nest or nest cluster was estimated by the surveyors (±1 m^2). Owing to the small size of the river, the low water discharge (~0.25 $m^3 \cdot s^{-1}$), and the clarity of the water, the whole riverbed was visible during the census. Areas containing nests were recognized by being lighter in colour than surrounding areas, as a result of the mechanical disturbance of female digging during nest construction. In cases where the visual identification of nests was uncertain, gravel was gently removed to check for the presence of eggs. Nest area is likely to provide a more accurate estimate of total egg deposition than the number of nests, since individual nests are hard to distinguish in high density spawning areas. Also, larger fish with a larger number of eggs tend to dig larger nests (Crisp and Carling 1989). Total area (±1 m^2) of disturbed gravel was, therefore, used as a proxy for egg deposition. In the River Børsa, both the spawning period and the size of brown trout and Atlantic salmon overlap, making it impossible to distinguish between nests made by the two species. Total nest area therefore includes both Atlantic salmon and brown trout.

Juvenile abundance and size

Densities of juvenile salmon and trout were surveyed during July 2007 by single-pass electrofishing of transects (~1.5 m wide) across the width of the river at regular 25 m longitudinal intervals throughout the study area, resulting in a total number of 124 transects. The 25 m intervals were measured with a tape measure and the position of each transect spatially referenced by GPS. Single pass approaches provide cost-effective estimates of spatial variation in abundance that correlate highly with those obtained from traditional, more accurate, but also more time-consuming multipass censuses (Prevost and Nihouarn 1999; Bateman et al. 2005; Reid et al. 2008). In areas of rapidly flowing water (i.e., riffles) a banner net of approximately 1 m^2 was used to catch any stunned fish drifting downstream, otherwise, two small dip nets were used. Juveniles observed escaping the area being fished were noted and added to the capture count. It was assumed that the ratio of salmon to trout of the observed fish was equal to that of the caught fish and the appropriate fraction of observed fish added to the catch. Within each transect the total number of YOY salmon (observed and caught) was divided by the area fished (wetted width (m) × 1.5 m) to provide relative density indices. Similar density indices were made for YOY trout and older juveniles (parr) of both species. A subsample of the captured YOY was measured (±1 mm fork length) and their size used as a proxy for growth.

Habitat survey

Habitat was quantified by measuring/classifying four different variables: (*i*) water depth, (*ii*) mesohabitat type, (*iii*) substrate class, and (*iv*) shelter availability. Water depth was measured every 1 m along each transect. Mesohabitat type was classified into "slow" (pools and walks) and "fast" (glides and riffles) for each individual transect, according to criteria in Borsanyi et al. (2004). To facilitate the efficient sampling of the large number of substrate measurements, commonly used substrate classification systems (e.g., Bain et al. 1985), which contain up to 15 different classes, were simplified into a system with 5 classes (1, sand < 2 cm; 2, gravel 2–12 cm; 3, cobble 12–30 cm; 4, boulders > 30 cm; and 0, bedrock), where class 2 represents typical spawning substrate for salmonids (Armstrong et al. 2003). The two most common (by area) substrate classes were visually estimated for each electrofishing transect. Interstitial shelter availability (i.e., number of interstitial spaces in the river bed > 3 cm deep) was measured according to Finstad et al. (2007), using a 13 mm rubber tube in three 0.25 m^2 sampling quadrats, which were equally spaced (i.e., one in the middle and two half way left and right of it)

along each transect. Values of both dominant and subdominant substrate classes within each transect were positively correlated with shelter availability ($r_{Spearman} = 0.49$; $p < 0.001$ and $r_{Spearman} = 0.33$; $p < 0.001$, respectively). A proxy variable representing substrate variation was therefore derived as the first principal component (PC1) from a principal component analysis of these three variables, explaining 89% of the variance (loadings PC1: shelter = –0.98, dominant substrate = –0.13, subdominant substrate = –0.12). Values of PC1 decreased with increasing substrate class and shelter availability.

Statistical analysis

Data from the spawning survey and data from transect electrofishing (including habitat measurements) were collated by transferring GPS references of transects and nests to ArcGIS (ESRI, Redlands, California). Around each transect, 12.5 m zones were created and total nest area within each zone was calculated. Thus, 25 m sections were created containing information on nest area (sum of whole section) and habitat variables and relative YOY abundance (measured at the transect in the middle of the section).

Kernel smoothing (Gaussian kernel type, bandwidth = 300 m) was applied to graphically illustrate the spatial trends of nest area, YOY density, and body length along the river. The effects of biotic and abiotic variables on YOY salmon density and growth were then determined using generalized additive models (GAMs). GAMs are semiparametric extensions of generalized linear models (GLMs), where the linear predictor has been exchanged for a smoothed, additive predictor. These models have been described as being data- rather than model-driven, as the relationship between response and predictor variables is determined by the data instead of an a priori assumed parametric function. Thus, the use of GAMs avoids making untested assumptions about the relationship between response and predictor variables (Hastie and Tibshirani 1990; Guisan et al. 2002). Predictors in the YOY salmon density model were (*i*) nest area (summed over 12.5 m up- and down-stream of each transect), (*ii*) habitat type (slow or fast), (*iii*) density of parr, (*iv*) mean depth, and (*v*) substrate and shelter PC1. Predictors for the growth model (with body length as the response variable, used as a proxy for growth) were the same as those for the density model, except that nest area was replaced by YOY salmon density as a predictor. GAMs were constructed using a forward stepwise procedure. Starting with a single predictor model for each measured variable, the model with the highest deviance explained (DE) was chosen as a basis for the next model. Then, in turn, each remaining predictor variable was added to the preceding model and the model with the highest DE was retained. Subsequently, all of the predictors were added in that manner and each new model was tested against the preceding model using Akaike's information criterion (AIC) until an optimal model was obtained. Goodness of fit between alternative models was then evaluated by comparing both DE and Generalized Cross Validation (GCV) scores (Craven and Wahba 1979). To establish a more accurate hierarchy of the terms used in the models, each predictor was in turn removed from the best fitting model and the change in DE checked against the original model. Thus the predictor whose removal caused the greatest change in DE was deemed the most influential. The mesohabitat index contained too few unique values for a smoothed function to be applied, and hence, was added as a parametric term. Transects with zero density, and hence, no length measurements, were omitted from the growth-models. Thus the analyses included a total of 123 and 112 transects for density- and growth-models, respectively.

Given the nature of the data, we are aware that our observations may be spatially autocorrelated, thereby potentially amplifying the significance of the model terms in the results. However, spatial autocorrelation is unlikely to cause a shift in the regression coefficients in designs where observations are equally spaced over the entire study area, and thus does not generate a systematic bias in model estimates (Hawkins et al. 2007). Our design, with 25 m between each transect, spreads any small-scale spatial autocorrelation (which may be introduced by adding adjacent observations) evenly over the full extent of the study site. Furthermore, autocorrelation plots revealed that GAM residuals were autocorrelated by no more than a lag of seven transects, thus making it highly unlikely that autocorrelation significantly influenced our results. Finally, because there was a clear spatial trend in the distribution of nests (Fig. 1*a*), including both transect number as a spatial component and nest area in the model would introduce problems of collinearity, which would influence both parameter estimates and *p* values (e.g., Kleinbaum et al. 1998, p. 238).

All analyses were completed using the statistical software package R 2.6.0 for Windows (R Development Core Team 2007). GAMS were from the mgcv library (Wood 2001). The degree of smoothness of model terms in GAMs from this library is estimated as part of the fitting. Smooth terms in the GAMs were penalized regression splines, where degrees of freedom were limited to four to avoid overfitting.

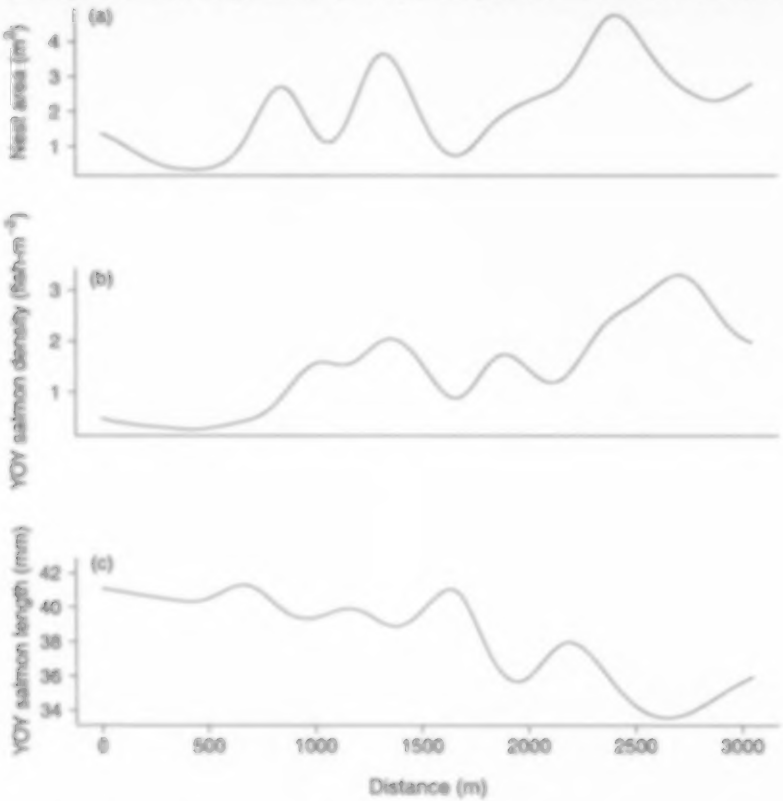
Results

The fish samples were dominated by salmon YOY and only ~6% of the captured juveniles were trout (no. trout YOY = 139, no. trout parr = 12), suggesting that trout spawning had a minor influence on the nest area and distribution data. Thus, trout data were not included in the analysis. A total of 2217 salmon YOY and 115 salmon parr were caught with a mean density of 1.48 fish·m^{-2} and 0.09 fish·m^{-2}, respectively. For salmon YOY, minimum and maximum length ranged between 27 mm and 55 mm (mean = 38 mm), and ranged between 65 mm and 131 mm (mean = 87 mm) for salmon parr. Approximately 86% of the fish included in the analysis were caught and handled, rather than observed and counted (no. observed YOY, 374; no. observed parr, 39). Nest area, YOY salmon density, and body length varied considerably along the study area (Fig. 1). Positive associations between nest area and YOY density, and negative associations between YOY density and body size were visually evident. A total of 283 spawning locations were identified, with a total area of 333 m^2 and a mean of 2.11 m^2 per transect.

Density

The optimal GAM model for YOY Atlantic salmon density explained 38.8% of the deviance (Table 1). Nest area as an index of total egg deposition had a strong positive in-

Fig. 1. Kernel-smoothed estimates of (*a*) nest area, (*b*) young-of-the-year (YOY) Atlantic salmon (*Salmo salar*) density, and (*c*) YOY salmon length in the River Børsa against distance along the river (zero representing the downstream end of the studied part of the river).



fluence (GAM: $F_{[-2.117]} = 25.85$, $p < 0.001$) on local densities of YOY Atlantic salmon (Fig. 2) and was the most important correlate of local densities, relative to the other variables measured (Tables 1 and 2). Habitat type also had a significant effect on YOY density (Table 1), where densities were highest in the fast flowing habitat category (GAM: $t_{[122]} = -3.47$, $p < 0.001$). Although including PC1 produced a better model fit based on AIC (Table 1), neither the substrate/shelter PC1 nor depth significantly affected YOY densities (GAM: $F_{[-2.114]} = 2.14$, $p = 0.116$ and $F_{[-1.116]} = 0.90$, $p = 0.356$, respectively) and hence were excluded from the model. The density of salmon parr was positively related to the density of YOY salmon (GAM: $F_{[-3.117]} = 3.94$, $p = 0.012$) and removing this term from the GAM resulted in significant loss of explanatory power (Table 2).

Growth

YOY salmon density was negatively correlated with YOY salmon length (Fig. 3) (GAM: $F_{[-2.106]} = 26.81$, $p < 0.001$; Figs. 3 and 4), which constituted the strongest predictor of YOY salmon growth, relative to the other variables measured (Table 2). Depth negatively influenced growth at low to intermediate depths (<60 cm) (GAM: $F_{[-2.106]} = 5.73$, $p = 0.003$; Fig. 4). At greater depths, the relatively few observations caused wide 95% confidence intervals of the GAM,

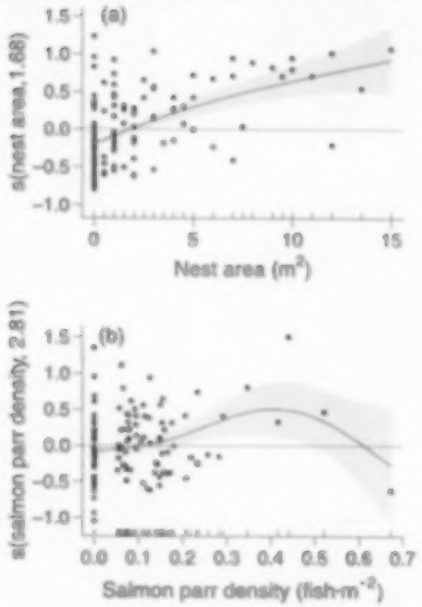
Table 1. Multiple predictor generalized additive model (GAM) fits for young-of-the-year (YOY) Atlantic salmon density and growth in the River Børsa.

Model		Deviance (%)	GCV score	AIC
Density				
1	NA	25.4	0.23669	173.76
2	NA+HT	33.2	0.21615	162.53
3	**NA+HT+PD**	**38.8**	**0.20674**	**156.84**
4	NA+HT+PD+PC1[a]	42.9	0.20144	153.33
5	NA+HT+PD+PC1+D[b]	43.3	0.20318	154.23
Growth				
1	SD	25.5	0.0061	–251.44
2	SD+PC1	38	0.00517	–269.88
3	**SD+PC1+D**	**45.4**	**0.00477**	**–279.19**
4	SD+PC1+D+PD	47.8	0.00478	–279.24
5	SD+PC1+D+PD+HT	47.9	0.00486	–277.67

Note: NA, nest area; SD, young-of-year salmon density; HT, habitat type; PD, salmon parr density; D, depth; PC1, substrate index and shelter principal component; GCV, generalized cross validation; AIC, Akaike Information Criterion. The best fitting model is given in bold.

[a]PC1 not significant.
[b]PC1 & D not significant.

Fig. 2. Effects of significant smoothed predictor variables (s) on the density of young-of-the-year (YOY) Atlantic salmon (*Salmo salar*) in the River Børsa, as estimated by generalized additive models (GAMs). The *y* axis represents the average contribution of the predictor variable using a logit scale. Zero on the *y* axis corresponds to no effect of the predictor variable at the corresponding value on the *x* axis. Parentheses on the *y* axis contain the degrees of freedom associated with the relevant predictor, and etchings (rugs) on the inside of the *x* axis indicate locations of data points along this axis. Shaded areas represent 95% GAM specific metric credibility intervals.



making the relationship between depth and growth difficult to ascertain. The substrate and shelter PC1 had significant negative influence on growth (GAM: $F_{[-1.936]} = 18.14$, $p < 0.001$; Fig. 4). Because the smaller PC1 scores represent large substrate and high shelter availability this implies that growth is positively related to substrate complexity and shelter. Neither the density of salmon parr (GAM: $F_{[-3.104]} = 1.65$, $p = 0.187$), nor habitat type (GAM: $t_{111} = 0.85$, $p = 0.395$) significantly affected the growth of YOY salmon and were consequently removed from the analysis. Thus the optimal model explained 45.4% of the deviance (Table 1).

Discussion

River-scale monitoring of the spatial distribution of nests and subsequent YOY densities and sizes provided strong evidence that spawning site distribution has significant effects on the growth of juvenile Atlantic salmon, via density dependence. These results support previous findings that patchily distributed spawning locations and limited dispersal capabilities of salmonid YOY give rise to variation in YOY salmon densities (Crisp 1995; Armstrong 2005; Einum et al. 2008*b*). More importantly, not only did the distribution of spawning sites significantly influence local densities, but relative to other measured variables the amount of nest area clearly proved to be the most important predictor.

Judging by the limited influence of other measured habitat variables, it appears that there was little redistribution of salmon YOY according to habitat preferences. Owing to our correlative approach, habitat variables not measured may well have influenced the distribution of YOY, with preferred habitats being located closer to spawning grounds than non-preferred ones. Furthermore, redistribution according to habitat preferences over smaller spatial scales (i.e., microhabitat) than those examined here would have been harder to detect. Nevertheless, our conclusion that distribution of YOY in natural populations is heavily influenced by nest distributions, is backed up by manipulative studies (Einum and Nislow 2005; Einum et al. 2008*b*), suggesting a causal relationship. Although the term related to salmon parr densities was retained in the density model on statistical grounds, a positive relationship between the two cohorts seems unlikely, as an antagonistic relationship is expected between age classes and species (Kaspersson and Höjesjö 2009). Instead it is more probable that certain habitats attracted larger numbers of parr in this system, hence resulting in the observed positive relationship. Further, it is possible that parr may have remained close to their native site, resulting in a positive correlation between fry and parr densities.

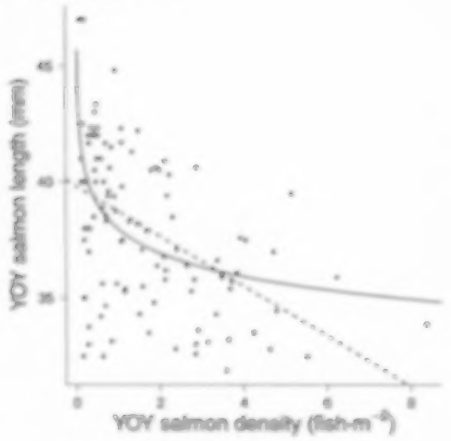
Salmon growth in the River Børsa was clearly density-dependent, the decline with density being equally well described by a negative power function, characteristic for stream dwelling salmonids (Grant and Imre 2005), or a linear regression. Again, most habitat variables, as well as varying densities of older cohorts, proved to have little effect on YOY salmon growth, and the density of conspecifics was likely a dominant influence. YOY salmon growth was positively related to low values of the substrate/shelter PC1 (negatively related to the substrate size and shelter availability), whilst there was no significant relationship between YOY salmon densities and PC1. In contrast, previous studies have linked higher YOY densities to increased habitat complexity, owing to decreased territory size (Dolinsek et al. 2007; Venter et al. 2008). However, these studies quantified habitat complexity at a finer scale, and may therefore be better able to detect relationships between habitat heterogeneity and fish densities. It has also been suggested that more complex habitats may enhance salmonid growth by providing foraging benefits and decreased metabolic cost (Millidine et al. 2006; Dolinsek et al. 2007; Venter et al. 2008). This supports the argument that while nests (and hence YOY densities) may be placed as independent of substrate complexity, emerging YOY may profit from structurally complex habitat owing to improved foraging opportunities and increased shelter availability.

While previous studies provide insights into relationships between the spatial distribution of spawning sites and the resulting juvenile densities and growth (Crisp 1995; Einum and Nislow 2005; Einum et al. 2008*b*), this is the first study demonstrating such a link on a large scale in a natural population. Moreover, we show that the relative importance of female nest placement for local YOY density and subsequent growth is significantly greater than that of local habitat conditions. Although our results are based on only one population in one year, combined with

Table 2. Ranking of terms based upon the change in percent deviation explained and generalized cross validation (GCV) scores from the relevant optimal fit model.

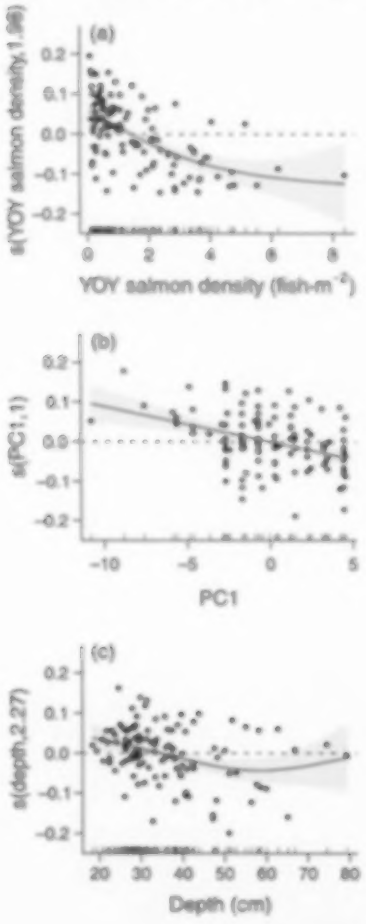
	Density		Growth	
Best fit model minus:	Δ%deviation explained	ΔGCV score	Δ%deviation explained	ΔGCV score
Nest area	25.2	–0.07711	—	—
YOY salmon density	—	—	29.7	–0.00226
Habitat type	8.2	–0.02225	—	—
Parr density	6.3	–0.01221	—	—
Depth	—	—	7.4	–0.00040
Substrate index and shelter principal component	4.1	–0.0053	9.7	–0.00071

Fig. 3. Average fork length of young-of-the-year (YOY) Atlantic salmon (*Salmo salar*) relative to density per transect in the River Børsa. Negative linear functions and nonlinear power functions provided equal descriptions of the data ($y = -1.047x + 39.811$, $t = 5.81$, $p < 0.001$, $r^2 = 0.23$, AIC = 571.86; $y = 37.858x^{-0.04}$, $t = -5.91$, $p < 0.001$, $r^2 = 0.24$, Akaike Information Criterion (AIC) = 570.89, respectively).



the results from the previously cited experimental studies (Crisp 1995; Einum and Nislow 2005; Einum et al. 2008*b*), the observed patterns may also apply to other populations. Insights from this current study present a shift from the commonly applied habitat simulation models (*e.g.*, PHABSIM; Bovee 1986). Such models assume that individuals actively choose their position in the stream, according to certain habitat attributes. Adding to the limitations inherent in these models (Mathur et al. 1985; Hudson et al. 2003; Railsback et al. 2003), our results imply that at least for salmon YOY, habitat selection and subsequent growth are largely determined by the location of the nest site. Therefore the presence of an individual at any given point in the stream may rather be the consequence of maternal nest selection instead of active YOY habitat selection. Further, these results suggest that the spatial distribution of potential spawning areas may be important for YOY growth and survival, potentially influencing population carrying capacity.

Fig. 4. Effects of significant smoothed predictor variables (s) on the length of young-of-the-year (YOY) Atlantic salmon (*Salmo salar*) in the River Børsa, as estimated by generalized additive models (GAMs). The *y* axis represents the average contribution of the predictor variable using a logit scale. Zero on the *y* axis corresponds to no effect of the predictor variable at the corresponding value on the *x* axis. Parentheses on the *y* axis contain the degrees of freedom associated with the relevant predictor and etchings (rugs) on the inside of the *x* axis indicate locations of data points along this axis. Shaded areas represent 95% GAM specific metric credibility intervals.



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An experimental examination of temperature interactions in the match–mismatch hypothesis for Pacific cod larvae

Benjamin J. Laurel, Thomas P. Hurst, and Lorenzo Ciannelli

Abstract: The match–mismatch hypothesis (MMH) predicts that marine fish larvae will have their highest rate of growth and survival when they overlap with their prey. However, Pacific cod (*Gadus macrocephalus*) and other gadids continue to thrive in the Bering Sea despite delayed prey production resulting from warming and loss of sea ice. In this study, we examined how temperature mediates growth and survival of Pacific cod larvae under varying match–mismatch conditions. Cod larvae were reared at two temperatures (3 °C and 8 °C) and exposed to one of five different food treatments over a 6-week period: (*i*) high food (HF) (match); (*ii*) low food (LF); (*iii*) HF then LF (HF–LF); and (*iv*) LF then HF (LF–HF) (2–4 mismatch); and (*v*) no food (NF) (complete mismatch). Results showed that cold environments allow Pacific cod larvae to bridge gaps in prey availability (i.e., timing and magnitude), but negatively impact survival over longer periods. Under warmer conditions, mismatches in prey significantly impacted growth and survival. However, both yolk reserves and compensatory growth mechanisms reduced the severity of mismatches occurring in the first 3 weeks of development. Our results demonstrate a clear need to incorporate the direct effects of temperature on fish larvae in food limitation models.

Résumé : L'hypothèse de l'appariement–mésappariement (MMH, match–mismatch hypothesis) prédit que les larves de poissons marins possèdent une croissance et une survie maximales lorsqu'il y un chevauchement entre elles et leurs proies. Cependant, la morue du Pacifique (*Gadus macrocephalus*) et d'autres gadidés continuent de prospérer dans la mer de Béring malgré la production retardée de proies causée par le réchauffement et la perte de glace de mer. Nous examinons dans notre étude comment la température contrôle la croissance et la survie de la morue du Pacifique sous diverses conditions d'appariement–mésappariement. Nous avons élevé des larves de morue à deux températures (3 °C et 8 °C) et les avons exposées à l'un de cinq traitements alimentaires durant une période de 6 semaines: (*i*) nourriture abondante (HF) (appariement), (*ii*) nourriture restreinte (LF), (*iii*) HF puis LF (HF–LF), (*iv*) LF puis HF (LF–HF) (2–4 mésappariements) et (*v*) absence de nourriture (NF) (mésappariement complet). Nos résultats montrent que les environnements froids permettent aux larves de morues du Pacifique de surmonter des interruptions dans la disponibilité des proies (c'est-à-dire le moment et l'intensité de la pénurie); mais cela affecte négativement la survie sur des périodes plus longues. Dans des conditions plus chaudes, les mésappariements des proies affectent significativement la croissance et la survie. Cependant, les réserves de vitellus et les mécanismes compensatoires de la croissance réduisent tous deux la sévérité des mésappariements qui se produisent durant les trois premières semaines du développement. Nos résultats démontrent clairement la nécessité d'incorporer les effets directs de la température sur les larves de poissons dans les modèles de restriction de nourriture.

[Traduit par la Rédaction]

Introduction

Climate-driven impacts on ocean environments are changing the seasonal distribution and timing of zooplankton production worldwide (Richardson 2008). Marine fish larvae, which depend on zooplankton for food, are highly vulnerable to such changes because they lack energetic reserves (Theilacker et al. 1996) and need sufficient food to grow fast and decrease their vulnerability to size-dependent predation (e.g., Pepin 1993). Therefore, spatial–temporal changes in zooplankton resulting from climate change may be impacting recruitment success in commercially important fish species (Beaugrand et al. 2003; Edwards and Richardson 2004). However, the effects of changing zooplankton patterns will likely vary across ecosystems, depending on species-specific physiology, local environmental conditions, and the degree of change in the timing/magnitude of prey production (Platt et al. 2003).

In the Bering Sea, changing ocean temperature and wind-forced mixing determine the annual variation in the south-

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B.J. Laurel[1] and T.P. Hurst. Fisheries Behavioral Ecology Program, Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, Hatfield Marine Science Center, Newport, OR 97365, USA.
L. Ciannelli. College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, OR 97331, USA.

[1]Corresponding author (e-mail: ben.laurel@noaa.gov).

erly extent of sea-ice, which in turn drive the magnitude and timing of productivity for pelagic and benthic communities in the spring (Hunt et al. 2002). Late ice retreat is coupled with an early bloom (March–April) in cold water, whereas early retreat of ice has resulted in a late bloom (May–June) in warmer water. Over the past two decades, increased ocean and air temperature have drastically reduced sea ice extent, and it is hypothesized that pelagic fish larvae are benefiting from "late bloom" conditions because zooplankton production can be >2× higher in magnitude in the warmer water (Hunt and Stabeno 2002; Napp et al. 2002). The recent increase in pelagic fish communities (e.g., walleye pollock, *Theragra chalcogramma*, and Pacific cod, *Gadus macrocephalus*) support this contention (Hunt et al. 2002). However, the larvae of these pelagic fish species are best temporally synchronised with the earlier, less productive blooms occurring in cold water (Hunt et al. 2002; Matarese et al. 2003), the ecological significance of which is unknown.

The match–mismatch hypothesis (MMH; Cushing 1975, 1990) is an existing model framework used to predict recruitment success of fish stocks as a result of annual variability in temporal/spatial overlap of fish larvae and their prey. The MMH assumes (*i*) that the spawning timing, and consequential peak abundance of larvae, is annually fixed in time whereas (*ii*) the spawning timing of phytoplankton (followed by zooplankton) is variable, owing to changing environmental conditions, i.e., temperature. The MMH has received renewed attention in a variety of ecosystems where changing oceanic temperatures have disrupted the phenological processes of predators and prey (Forchhammer et al. 1998; Anderson and Piatt 1999; Durant et al. 2007). However, fish are poikilotherms, and the MMH framework does not consider how temperature may directly impact the metabolic demands of larvae under changing food conditions. For example, the energetic demands of fish larvae increase under warm conditions, thereby reducing the temperature for optimal growth and survival in food-limited situations (Otterlei et al. 1999; Buckley et al. 2004). Similarly, match–mismatch conditions may have little consequence for fish larvae at colder temperatures because of temperature-limited growth (Jobling 1988). Based on these principles, the role of temperature has been hypothesized to be important in match–mismatch situations (e.g., Beaugrand et al. 2003), yet to date, the MMH has only been experimentally examined under a single temperature scenario (Gotceitas et al. 1996; Bos et al. 2006).

In this study, we experimentally test the MMH at two temperatures (3 °C and 8 °C) using Pacific cod larvae. Pacific cod are highly fecund (up to 5 000 000 eggs; McCain 2003), but spawn semi-demersal eggs that are released in one batch during the spring spawning season in Alaskan waters (Mecklenburg et al. 2002) before moving into upper surface waters as larvae (Hurst et al. 2009). The relatively discrete spawning characteristics of Pacific cod, both temporally (i.e., single batch-spawning) and spatially (i.e., reduced dispersal potential of eggs), may make them particularly sensitive to spatial and temporal changes in productivity in the Bering Sea. We use our experimental results to determine whether the predictions of MMH are equally valid under cold and warm conditions while simultaneously providing the first vital rate data for Pacific cod larvae from the laboratory.

Materials and methods

Egg collections/rearing

Fish for larval experiments were reared in the laboratory from eggs collected from spawning adults. In April 2007, female and male Pacific cod were caught by commercial jigging gear from spawning grounds in Chiniak Bay, Kodiak Island, Alaska, USA. The gametes were mixed and placed into 4 L incubation trays at 4 °C. At 24 h post-fertilization, fertilized eggs were shipped in insulated containers filled with 4 °C chilled seawater to Alaska Fisheries Science Center (AFSC) laboratory facilities in Newport, Oregon. Eggs were transferred to flow-through 4 L plastic trays and incubated at 4 °C until hatching. Hatching occurred 19–22 days post-fertilization, after which larvae were transferred into 100 L upwelling cylindrical tanks. Light regime during larval rearing was maintained at 12 h light : 12 h dark, with light provided by overhead fluorescent bulbs at a level of 6.7 μmol photons·m^{-2}·s^{-1} at the water surface.

Experimental design

The larval feeding experiment was carried out in 100 L conical upwelling fiberglass tanks with dark green interiors. Tanks were kept at the 12 h : 12 h photoperiod to approximate day-length conditions experienced by cod larvae in the Gulf of Alaska in March–April. Water was supplied at a rate of 250 mL·min^{-1} through a centre-bottom intake to minimize disturbance to the larvae. Gentle aeration was provided by an airstone to provide additional circulation in the tanks.

Tanks were maintained at two temperatures (3 °C and 8 °C) to reflect the conditions at early and late times of the year, respectively. A priori, we recognized that it would be logistically too difficult to replicate the range of prey types and densities representative of variable field conditions during these periods. Therefore, our experiment focused on varying relative proportions of nutritionally enriched prey to examine the effects of food availability (i.e., match or mismatch) on growth and survival of the larvae. At both temperatures, larvae were exposed to five prey treatments over a 6-week period following feeding treatments established by Gotceitas et al. (1996) for use in Atlantic cod larvae. These consisted of (*i*) high food throughout (HF; "match"); (*ii*) low food throughout (LF); (*iii*) HF initially, then LF (HF–LF); (*iv*) LF initially, then HF (LF–HF) (*ii*, *iii*, and *iv* partial mismatch); and (*v*) no food (NF) (control and complete "mismatch"). The timing of the switch between food treatments (i.e., LF–HF and HF–LF) was set at the end of week 3. For reference, Pacific cod larvae are incapable of surviving beyond week 3 in the complete absence of food at 4 °C (Laurel et al. 2008).

Food consisted of a combination of rotifers (*Brachionus plicatilis*) and microparticulate dry food (Otohime A, Reed Mariculture Inc.). Rotifers were enriched with Algamac 2000, an enrichment containing high percentages of essential long-chained fatty acids known to be important for high growth and survival in Pacific cod larvae (Copeman and Laurel 2010). For HF and LF treatments, rotifers were supplied at a density of 4 prey·mL^{-1} and 0.5 prey·mL^{-1}, respectively, twice daily. These densities were chosen because preliminary feeding trials indicated they can sustain Pacific cod larvae at 8 °C, and the range in prey concentrations are

known to influence growth rates in similarly sized Atlantic cod larvae (Puvanendran and Brown 1999). Tanks were clear of rotifers after ~2 h, indicating that all prey were either consumed and (or) flowed out the tank between feedings. This "pulse feeding" design ensured that larvae were feeding on newly enriched prey, and that prey quality did not deteriorate over the course of the experiment. The Otohime microparticulate dry food was added to tanks twice daily at similar ratios (LF 0.3 g·tank^{-1} once daily; HF 0.6 g·tank^{-1} twice daily) as an additional supplement to the rotifer diet. The microparticulate diet was used because it was larger in diameter than mean rotifer length (220–350 μm) and this factor was anticipated to be important for faster growing fish as they switched to the preferred larger prey sizes.

Three replicate tanks were used for each temperature – feeding treatment combination (n = 30 tanks). A total of 500 larvae were added to each tank. To facilitate the monitoring of daily mortality, the NF control treatments were conducted in smaller (1 L) containers (n = 100 larvae) nested in the larger tank. This allowed for the measurement of daily mortality by siphoning the bottom of the containers each day and counting the number of dead larvae.

Larvae were sampled at 1-week intervals from each experimental tank (n = 7–10 larvae per tank), starting with "Day 0" post-hatch when larvae were first introduced to the experimental tanks (n = 20 larvae total). An image analysis system was used to make the following morphometric measurements on living larvae: (*i*) standard length (SL) in millimetres, (*ii*) body depth at the anus (BD) in millimetres, (*iii*) eye diameter (ED) in millimetres, and (*iv*) yolk area (YA) in square millimetres. No morphometric data were recorded in the NF control treatments — these treatments were specifically designed to examine the duration at which larvae could survive in the complete absence of food. After measurements were complete, larvae were transferred to separate rearing tanks and no longer used for the remainder of the experiment. Survival in the feeding treatments (i.e., HF, HF–LF, LF–HF, LF) was measured at the end of the experiment by siphoning and counting individual larvae out of each tank.

The degree of gut fullness was also quantified on a scale of 0 to 3, with 0 indicating empty and 3 indicating full. Dry mass (DM) of individual larvae was calculated using a 2-step model based on SL and BD:

$$(1)\quad \mathrm{DM} = 0.020346 \times e^{(0.373366 \times \mathrm{SL})} + (-0.111299 + 0.968468 \times \mathrm{BDdev})$$

where BDdev was calculated using the following:

$$(2)\quad \mathrm{BDdev} = \mathrm{BD} - [0.0873 \times e^{(0.2164 \times \mathrm{SL})}]$$

The model was derived from a similar size range of Pacific cod larvae (0–45 days post hatch, dph) in a separate experiment and was able to explain >98% of DM variance in larvae using SL and BD measures. From the DM data, we calculated specific growth rate (SGR) using the following formula:

$$\mathrm{SGR} = (\ln \mathrm{DM}_{t2} - \ln \mathrm{DM}_{t2}) \times (t_2 - t_1)^{-1} \times 100$$

where DM_{t1} and DM_{t2} are the mean dry mass of cod larvae at times t_1 and t_2, respectively.

Data analysis

All statistical analysis and data plots were conducted on means from the experimental tank collected on each sampling date. The effects of temperature, food treatment, and larval age (weeks) on SL, DM, YA, SGR, and gut fullness were analyzed using analysis of variance under the general linear model (GLM). All residuals were checked to ensure data met the assumptions of normality. Plots of SL, DM, YA, and gut fullness were used in conjunction with the statistical model to discuss the nature of significant interactions revealed in the model. For SGR, statistical models were constructed to examine response variable effects prior to the food switch (i.e., 0–3 weeks post-hatch) vs. after the food switch i.e., 4–6 weeks post hatch. Pairwise comparisons (i.e., Tukey's honestly significant difference (HSD) tests) were additionally used in the analysis of SGR to make subsequent comparisons of larval performance among food treatments and sampling week, i.e., week 3 and week 6.

The effects of food-treatment and temperature on survival were examined at the end of the experiment (week 6). Data were examined non-parametrically using a Kruskal–Wallis one-way analysis of variance by ranks.

Results

The GLM revealed a significant three-way interaction between prey level, week (i.e., age post-hatch), and temperature for DM ($F_{[3,93]}$ = 3.10, p < 0.001; Fig. 1) of cod larvae (see supplementary data,[2] Table S1, for full model results). For SL, the GLM three-way interaction term was not significant, but instead revealed a series of significant two-way interactions between prey level × week ($F_{[15,93]}$ = 3.24, p < 0.001), week × temperature (GLM, $F_{[3,93]}$ = 77.15, p < 0.001), and prey × temperature (GLM, $F_{[3,93]}$ = 10.01, p < 0.001; Fig. 2; see supplementary data,[2] Table S2, for full model results). Nevertheless, the patterns in larval performance reflected in SL and DM were the same among treatment combinations (Figs. 1 and 2). That is, all larvae increased in SL and DM over time, but overall, larvae reared at 8 °C increased in size at a more rapid rate than larvae in the 3 °C treatment. The effects of prey treatment were also more apparent in larvae reared at 8 °C. Despite this, the effect of prey treatment ranked similarly within each temperature, with larvae in HF and the LF–HF treatments being the largest in terms of SL and DM at the end of the experiment, followed by larvae in the HF–LF, and finally the larvae in the LF treatment.

The GLM analysis of DM of larvae indicated there was a significant effect of prey treatment at week 6 at both temperatures (GLM, 3 °C: $F_{[3,8]}$ = 4.127, p = 0.010; 8 °C: $F_{[3,8]}$ = 22.267, p < 0.001), but at week 3, differences among prey treatment were only detected in the 8 °C treatment (GLM, 3 °C: $F_{[3,8]}$ = 1.561, p = 0.078; 8 °C: $F_{[3,8]}$ = 3.609, p = 0.017). To examine the effects of temperature and prey treatment simultaneously, a separate model was run for multiple comparison post-hoc analysis of DM among prey levels

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

Fig. 1. Changes in the dry mass of *Gadus macrocephalus* larvae over time when reared at (*a*) 3 °C and (*b*) 8 °C under four different prey treatments (HF, high food throughout (filled circle, ●); LF, low food throughout (open triangle, △); HF–LF: HF for the first 3 weeks, then LF (open circle, ○); LF–HF: LF for the first 3 weeks, then HF (filled inverted triangle, ▼)). Top and bottom panels represent larvae reared at 3 °C and 8 °C, respectively. The broken line indicates the timing in the change of food level in the HF–LF and LF–HF prey treatments. Values represent tank means ($n = 3$) ± 1 SE.

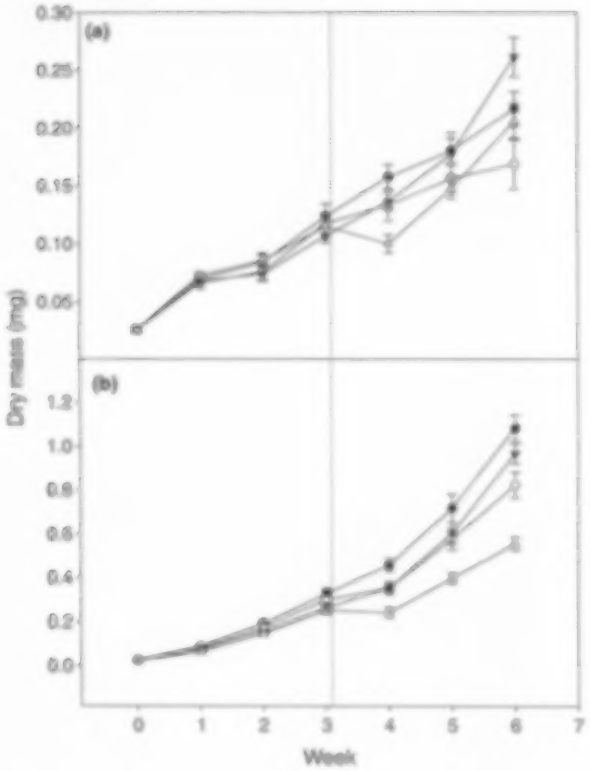
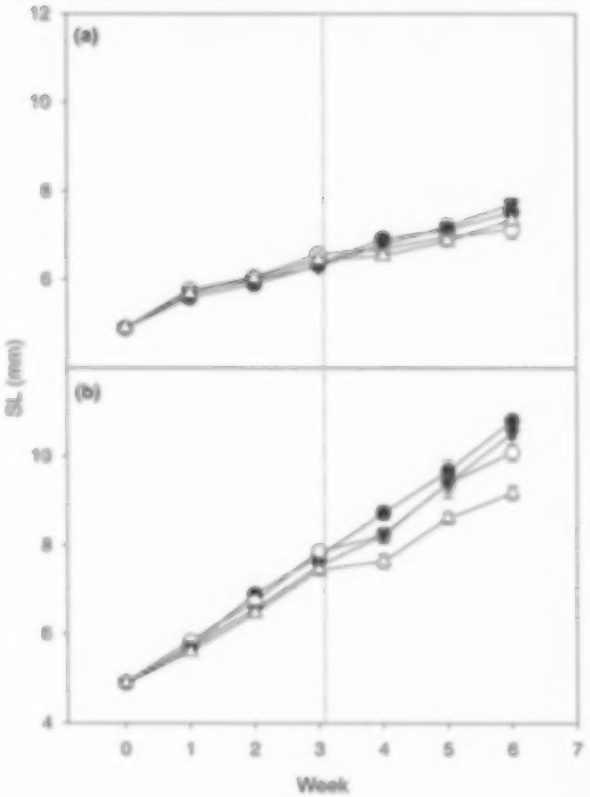


Fig. 2. Changes over time in the standard length (SL; mean ± SE) of *Gadus macrocephalus* larvae reared at (*a*) 3 °C and (*b*) 8 °C under four different prey treatments (HF, high food throughout (filled circle, ●); LF, low food throughout (open triangle, △); HF–LF, HF for the first 3 weeks, then LF (open circle, ○); LF–HF, LF for the first 3 weeks, then HF (filled inverted triangle, ▼)). Broken line indicates the timing in the change of food level in the HFLF and LFHF prey treatments. Values represent tank means ($n = 3$) ± 1 SE.



and temperature treatments by week (week 3 and week 6; See Tables 1 and 2). The results of the multiple comparisons indicate that the effects of temperature on larval mass were more extensive and occurred earlier in the experiment than the effects of prey treatment. For example, at week 3 and week 6, larvae had significantly higher mass in 100% of the 8 °C prey treatment comparisons with 3 °C prey treatments (Table 1). In contrast, within a temperature treatment, the effects of prey treatment were only significant in 17% and 42% of the comparisons at week 3 and week 6 of the experiment, respectively (Table 1 and 2). Most of these significant differences among prey treatments were in the 8 °C treatment. At week 6, larvae at 8 °C were significantly larger in the HF, HF–LF, and LF–HF prey treatments compared with larvae in the LF treatment, whereas at 3 °C, DM differences were only detected between the HF–LF and LF–HF treatment (Table 2).

Results are shown for the model in which growth (SGR) was compared between 0–3 weeks post-hatch and 4–6 weeks post-hatch as a function of food treatment and temperature (Table 3). As expected, growth was significantly slower at 3 °C compared with 8 °C, and this was reflected in the significant interaction term between temperature and time period (GLM, $F_{[1,30]} = 6.784$, $p = 0.014$; Table 3). At both temperatures, larvae in the HF treatment were growing faster than larvae in the LF treatment both in the 0–3 week and 4–6 week time period. However, the relative difference in larval growth exposed to these food treatments was notably less at cold temperatures (as indicated by the slopes in Fig. 3). Also, there was a significant interaction between time period and the prey treatment (Table 4; Fig. 3). At both temperatures, the slope in the LF–HF treatment is shallower than the other food treatments; the switch to HF after week 3 resulted in relatively higher growth than other food treatments. In fact, highest growth after week 3 was observed in the LF–HF treatment, suggesting compensatory growth may be occurring under such conditions.

Overall survival was sufficient to sample larvae to week 6 in all experimental treatments, with the exception of the NF treatment in which all fish larvae died at 12 dph and 22 dph

Table 1. Week 3 Tukey's honestly significant difference tests comparing larval dry mass values among 3 °C and 8 °C prey treatments.

		3 °C				8 °C			
		HF	HF–LF	LF–HF	LF	LF	LF–HF	HF–LF	HF
8 °C	HF	0.207*	0.183*	0.224*	0.216*	0.077*	0.065*	0.028	
	HF–LF	0.179*	0.155*	0.195*	0.187*	0.049	0.037		
	LF–HF	0.142*	0.118*	0.159*	0.151*	0.012			
	LF	0.130*	0.106*	0.147*	0.139*				
3 °C	LF	–0.009	–0.033	0.008					
	LF–HF	–0.017	–0.041						
	HF–LF	0.024							
	HF								

Note: Reported values are relative differences of the left column vs. upper column treatments; *, $p < 0.05$. HF, high food throughout; LF, low food throughout; HF–LF, high food for the first 3 weeks, followed by low food; LF–HF, low food for the first 3 weeks, followed by high food.

Table 2. Week 6 Tukey's honestly significant difference tests comparing larval dry mass values among 3 °C and 8 °C prey treatments.

		3 °C				8 °C			
		HF	HF–LF	LF–HF	LF	LF	LF–HF	HF–LF	HF
8 °C	HF	0.868*	0.915*	0.823*	0.879*	0.527*	0.117	0.199*	
	HF–LF	0.669*	0.717*	0.624*	0.680*	0.328*	–0.082		
	LF–HF	0.751*	0.798*	0.706*	0.762*	0.410*			
	LF	0.341*	0.389*	0.296*	0.352*				
3 °C	LF	–0.011	0.037	–0.056					
	LF–HF	0.045	0.012*						
	HF–LF	–0.048							
	HF								

Note: Reported values are relative differences of the left column vs. upper column treatments. *, $p < 0.05$. HF, high food throughout; LF, low food throughout; HF–LF, high food for the first 3 weeks, followed by low food; LF–HF, low food for the first 3 weeks, followed by high food.

Table 3. Specific growth rate comparison of Pacific cod larvae reared under differing prey treatments (HF (high food); LF (low food); HF–LF; LF–HF) at two time periods ("early" (0–21 days post-hatch) and "late" (22–42 days post-hatch)).

Source	df	*F* ratio	*p* value
Time	1	314.123	<0.001
Prey	3	2.816	0.069
Temperature	1	116.528	0.000
Time × prey	3	4.369	0.011
Time × temperature	1	6.784	0.014
Prey × temperature	3	1.412	0.259
Time × prey × temperature	3	1.092	0.368
Error	32		

at 8 °C and 3 °C, respectively. In the feeding treatments, survival was significantly higher at 8 °C than 3 °C at the end of the experiment (one-way Kruskal–Wallis test, $H = 10.517$, $p = 0.001$; Fig. 4). Analysing the data by temperature treatments separately revealed that survival ranked similarly among food treatments; the highest survival was observed in the HF treatment, followed by the LF–HF, HF–LF, and LF treatments (Fig. 4). However, there was no statistically significant effect of food treatment on survival (one-way Kruskal–Wallis test, 3 °C, $H = 0.691$, $p > 0.05$; 8 °C, $H = 4.351$, $p > 0.05$), likely due to the high variation among replicate tanks within each treatment.

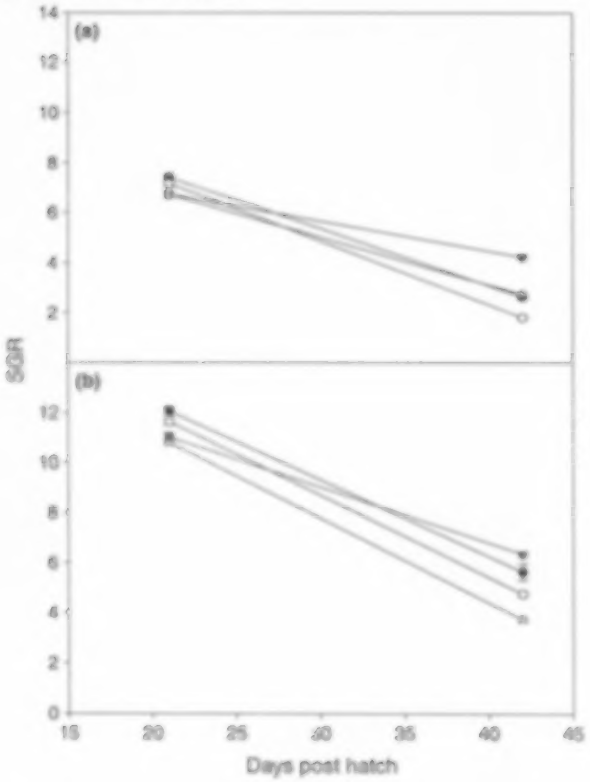
Food treatment appeared to have no effect on the maintenance of yolk in the Pacific cod larvae. However, as predicted, there was a strong interaction between larval age (week) and temperature on YA (GLM, $F_{[8,118]} = 6.477$, $p < 0.001$; see supplementary data,[2] Table S3, for full model results). Larvae in the colder temperature treatment were also able to survive longer in the absence of food, as indicated in the NF control treatments (Fig. 5). At 3 °C, 50% and 100% mortality occurred at 17 dph and 22 dph, respectively, whereas at 8 °C, 50% and 100% mortality occurred at 9 dph and 12 dph, respectively.

Prey were observed in the guts of larvae during the first week of sampling in all temperatures and food treatments where prey were present. The model results examining gut fullness as a function of week, food treatment, and temperature are provided (Table 4). Significant interactions were detected between temperature and week (GLM, $F_{[5,93]} = 4.511$, $p < 0.001$) and the food treatment and week ($F_{[15,93]} = 1.888$, $p = 0.021$). These effects were driven by an overall trend of increasing gut fullness with age but slightly lower fullness in the cold temperature treatment (Fig. 6). In addition, larvae exposed to HF almost always had higher gut fullness relative to LF treatments, whereas larvae exposed to switching food treatments (i.e., HF–LF or LF–HF) appeared to show either an increase (i.e., LF–HF) or decrease (i.e., HF–LF) following the changing food availability after week 3 (Fig. 6).

Discussion

The results of our experiment support the basic predictions outlined in the MMH. The best match conditions (i.e.,

Fig. 3. Changes in specific growth rate of *Gadus macrocephalus* larvae at 0–21 days post-hatch (dph) and 22–42 dph when reared at (*a*) 3 °C and (*b*) 8 °C under four different prey treatments (HF, high food throughout (filled circle, ●); LF, low food throughout (open triangle, △); HF–LF, HF for the first 3 weeks, then LF (open circle, ○); LF–HF, LF for the first 3 weeks, then HF (filled inverted triangle, ▼)). Food levels were switched in the HF–LF and LF–HF prey treatments after 21 dph.



the HF treatment) resulted in highest overall growth and better-surviving larvae than larvae exposed to prolonged mismatched conditions, i.e., LF treatments. However, our experimental results extend beyond the core predictions of the MMH by demonstrating that interactions of temperature and timing of match–mismatch can be potentially more limiting to growth and survival of Pacific cod larvae. For example, in our low temperature treatment, the complete mismatch in food (NF treatment) allowed larvae to maintain yolk reserves and survive longer than larvae exposed to higher temperatures. However, when exposed to either LF or HF concentrations of prey, larvae reared at high temperatures grew significantly faster and had better survival than larvae in the cold temperature treatment. We discuss these match–mismatch scenarios as a function of temperature and timing separately and summarize them together in the context of changing environmental and prey conditions in the North Pacific.

Timing of prey availability

The MMH assumes that the most important factor controlling larval mortality is the degree to which marine larvae and their prey are synchronized. Our results indicate that timing does indeed regulate survival and growth. In the field, the emergence of marine fish larvae often precedes the peak prey abundance, a pattern that models have confirmed as being optimal for rapid growth (Bollens et al. 1992). We observed a similar pattern in our data. The early mismatch (LF–HF treatment) had relatively little impact on the size of fish at the end of the experiment. When match conditions were restored from the initial mismatch, Pacific cod larvae demonstrated the ability to accelerate their growth to the point that growth trajectories approached convergence with the HF treatment. Such compensatory growth has been observed in other species, both in individually and group-housed experiments, and is sometimes accompanied by a hyperphagic response if prey levels are sufficiently high (Ali et al. 2003). In our experiment, gut fullness levels increased steadily with age in all treatments, but increased further following the prey switch in the LF–HF treatment after week 3. Although some increases in gut fullness could be related to changing larval size, the immediate response in gut fullness during the prey switch suggests the increased foraging response is size-independent. The consequences of increased growth (e.g., predator risk or future swimming

Table 4. Gut fullness comparison of Pacific cod larvae reared under differing prey treatments (HF (high food); LF (low food); HF–LF; LF–HF) over a 6-week period at two temperatures (3 °C and 8 °C).

Source	df	*F* ratio	*p* value
Prey	3	14.15	<0.001
Week	5	41.664	<0.001
Temperature	1	76.794	<0.001
Prey × week	15	1.888	0.021
Prey × temperature	3	1.456	0.225
Week × temperature	5	4.511	<0.001
Week × prey × temperature	15	1.399	0.140
Error	93		

Fig. 4. Percentage survival of *Gadus macrocephalus* larvae at week 6 post-hatch at 8 °C (black bars) and 3 °C (gray bars) in HF, LF, HF–LF, and LF–HF prey treatments (HF, high food throughout; LF, low food throughout; HF–LF, HF for the first 3 weeks, then LF; LF–HF, LF for the first 3 weeks, then HF). Values are tank means (n = 3) ± 1 SE).

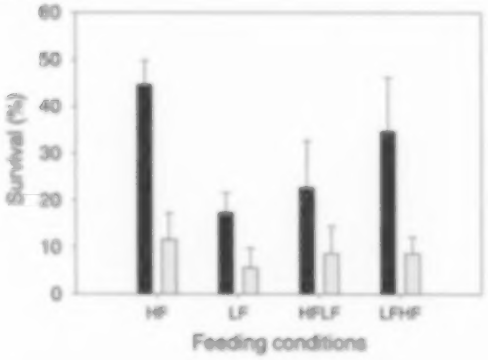


Fig. 5. Data for *Gadus macrocephalus* larvae showing that they survive (×) and maintain yolk reserves (filled circle, ●) longer at (*a*) 3 °C than (*b*) 8 °C. Survival data are taken from larvae reared in the absence of prey i.e., NF (no food) treatment; tank means (n = 3) ± 1 SE. Yolk reserves (area measures) are based on tank means (n = 12) ± 1 SE of larvae (n = 7–10 per tank) reared in combined prey treatments tanks (HF (high food); LF (low food); HF–LF; LF–HF) for each temperature.

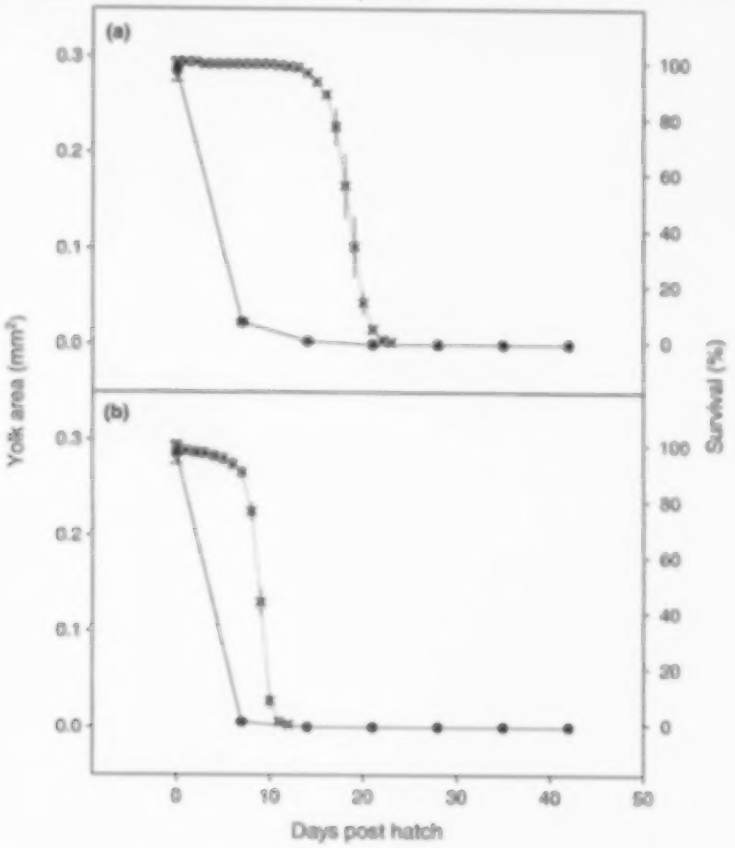
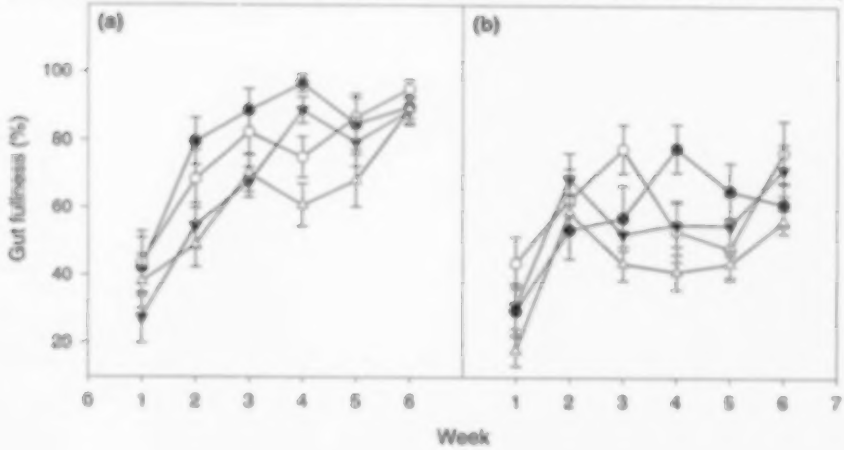


Fig. 6. Gut fullness of *Gadus macrocephalus* larvae at (*a*) 8 °C and (*b*) 3 °C by prey treatment (HF, high food throughout (filled circle, ●); LF, low food throughout (open triangle, △); HF–LF, HF for the first 3 weeks, then LF (open circle, ○); LF–HF, LF for the first 3 weeks, then HF (filled inverted triangle, ▼)) over time. Values are based on tank means (n = 3 ± 1 SE) of larvae (n = 7–10 per tank).



performance, development, and growth) were not evaluated in this study, so we cannot assume that compensatory growth is a cost-free mechanism for these larvae. However, it demonstrates that Pacific cod larvae have the potential for fast growth to offset historical periods of low growth potential when the environment is suboptimal e.g., prey mismatches.

There was very little difference in the size of larvae at week 3 among food treatments, lending further evidence that a temporal mismatch in prey during the early part of development is less consequential than a late mismatch. Likely, initial yolk reserves of larvae, depending on temperature, buffer the effects of low food concentrations during this period. An alternative explanation is that the smaller marine larvae put less demand on the standing crop of prey, thereby effectively making more prey available to larvae during the early part of the experiment. While marine larvae can locally deplete prey patches, which affects the survival and condition of the marine larvae (Duffy-Anderson et al. 2002), several lines of evidence make us doubt the relevance of this latter explanation. First, aquaculture studies with marine fish larvae generally agree that higher prey concentrations are required during the early ontogeny, mostly because of underdeveloped sensory and foraging capabilities, i.e., visual field, swimming speed, prey handling, etc. (Puvanendran and Brown 1999). Second, our experimental tanks were maintained at flow rates to "pulse feed" tanks, rather than attempt to sustain a targeted prey concentration over the course of a day, making it unlikely that larval feeding rates would be high enough to substantially impact prey biomass in the tank.

The potential impact of match–mismatch scenarios on recruitment dynamics is largely dependent on how discrete spawning is over time. A protracted spawning period can reduce interannual recruitment variability in Atlantic cod, presumably because mismatches in prey productivity potentially affect only a small proportion of the population (Mertz and Myers 1994). Our experiment focused on the effects of match–mismatch on a single batch of eggs spawned at one particular time in a range of possible times in the spawning season. Therefore, we cannot make predictions on the fate of an entire cohort or population level response to changing match–mismatch conditions. Rather, our results should be considered a guide in understanding cohort survival depending on the relative timings of peak larval emergence and prey production.

Temperature effects

The single most important factor regulating metabolic processes and growth rates in fish is considered to be temperature (Brett 1979; Houde 1989). The influence of temperature on the growth in gadids (e.g., Atlantic cod) is much greater during the early development (Björnsson and Steinarsson 2002), but to date these rates have just begun to be measured experimentally for Pacific cod larvae and juveniles (Hurst et al. 2010). As expected, there was a strong positive effect of temperature on growth, with a ~60%–70% higher mass-derived SGR observed in larvae at 8 °C vs. 3 °C throughout the experiment. Similarly, survival was ~2–4× higher among the warm temperature treatments. In contrast, prey availability, despite having a significant effect on the growth of larvae in both temperature treatments, explained less of the growth variation than temperature, overall. Temperature interacted with food availability, by magnifying growth and size differences among prey treatments at 8 °C and making such food-limited growth statistically evident earlier in the experiment (i.e., week 3) than larvae in the cold temperature treatments. However, the fact that larvae in the LF treatment at 8 °C outperformed the HF treatment at 3 °C indicates that temperature was the most limiting factor for growth and survival in our study.

Despite an overwhelming temperature effect, the role of food-limited growth has not been fully explored in our study. It was evident that the potential consequences of food-limited growth increased considerably from 3 °C to 8 °C in Pacific cod larvae. In Atlantic cod and haddock larvae, field data suggests that food limitation increases over a similar temperature range (Buckley et al. 2004). In the North Pacific, walleye pollock larvae suffer decreased growth, condition, and survival in the absence of abundant prey at ~4 °C (Canino et al. 1991; Bailey et al. 1995; Duffy-Anderson et al. 2002). Undoubtedly, the use of higher temperature treatments would illustrate this point further as the thermal tolerance of the species is approached and ration levels are unable to keep up with basal metabolic function (Jobling 1988). Such patterns have been demonstrated in the laboratory in other marine fish larvae over broader temperature ranges, but generally at temperatures exceeding those experienced in the field (Yin and Blaxter 1987; Otterlei et al. 1999). The temperatures used in our study fall within the range of what larvae may experience in the surface waters in the Bering Sea (2–6 °C) or in the Gulf of Alaska (4–8 °C) in late spring or early summer (Overland et al. 1999; Stabeno et al. 2004). Therefore, exploring growth at higher temperatures ranges, while demonstrating an increased relative effect of food- vs. temperature-limited growth, may have little relevance for modeling scenarios in the field.

Another way to examine food-limitation under the existing temperature treatments would be to further manipulate food rations. The prey concentrations used in our experiment were chosen because Pacific cod larvae were able to survive past flexion on both 0.5 prey·mL^{-1} and 4 prey·mL^{-1} in the laboratory during preliminary trials. It is likely that there is a ration level somewhere between 0–500 prey·L^{-1} that would have sustained Pacific cod larvae at 3 °C but would have been either unsustainable for larvae at 8 °C, or produced a broader range of growth differences. However, using similarly enriched prey at 8–9 °C, Atlantic cod larvae suffer high mortality at prey levels <0.5 prey·mL^{-1} and see little benefit in terms of growth beyond 4 prey·mL^{-1} (Puvanendran and Brown 1999). Therefore, it is likely our food treatments approached the lowest and highest to express the maximum growth range for Pacific cod larvae at 8 °C in our experiment, and by further manipulating food levels, there is likely little potential to produce a scenario in which growth rates in the HF treatment at 3 °C would equal, or outperform the LF treatment at 8 °C. Regardless, our goal was to demonstrate relative changes in growth and survival under highly contrasting match–mismatch scenarios, and by using prey levels that allowed for differential growth but adequate survival, the goals of the experiment were achieved.

It is important to emphasize that while warm conditions are favorable for growth and survival, there is an overall reduced duration in which larvae can survive in the complete absence of food. Larvae in our experiment were able to survive nearly twice as long at 3 °C compared with 8 °C when prey were unavailable. Factoring in the advantage of temperature-mediated yolk catabolism, favorable habitat for newly hatched Pacific cod larvae has been shown to increase in cold regions of the North Pacific when prey availability is low (L. Ciannelli, unpublished data). However, in the presence of prey levels adequate for growth, there was a strong, positive correlation between growth and survival in our experiment. Given that temperature explained the most growth variation, the greatest variance in survival was observed between the cold (low survival) and warm (high survival) treatments. Larval mortality rates typically follow a Type II functional response (Houde 1989), and the delayed development of larvae in cold-temperature and (or) the food-limited treatments may have driven the mortality higher as a consequence. The dramatic decrease in survival at low temperatures suggests that metabolic limitations (e.g., food assimilation, decreased membrane fluidity) may also be a factoring issue (Sinensky 1974). Neither Pacific nor Atlantic cod can be kept at size on a maintenance diet during the larval period, suggesting marine gadids must continually "grow or die" as larvae during the first 8 weeks of development (B.J. Laurel, personal observation). Elsewhere, it has been shown that larvae that develop faster and metamorphose at an earlier age typically have lower mortality rates than individuals remaining longer in the larval period (Chambers and Leggett 1987). Because larvae were not exposed to predators during our experiment, we attribute the positive correlation of growth and survival to be due to the increased foraging ability and consumption potential of older, more well-developed larvae (Pepin 1991; Bochdansky et al. 2008). Therefore, the challenge will be to determine whether the short-term survival benefits of cold-water (e.g., prolonged yolk reserves) outweigh the potential long-term impacts on survival, as observed directly (in this experiment) and possibly indirectly through increased stage-duration mortality.

Temperature and prey production

From our data, it is clear that temperature can reduce (cold conditions) or magnify (warm conditions) mismatch situations for Pacific cod, but ultimately Pacific cod lack the energetic reserves needed to survive long periods of compete prey absence. Therefore it is difficult to predict how Pacific cod larvae respond to patterns of delayed productivity in Alaskan waters. This is further complicated by the fact that temperature also regulates the timing and magnitude of prey production in Alaskan waters. For example, in the Bering Sea, warm conditions results in early sea ice retreat but delays productivity to late spring when waters can stratify (Hunt et al. 2002). Although the magnitude of this productivity tends to be much higher because it occurs in warmer water, Pacific cod larvae have insufficient yolk reserves to take advantage of such production if peak larval supply precedes the bloom by more than 2–3 weeks. Even under extreme cold conditions (e.g., 0 °C), Pacific cod larvae are unable to survive past 4 weeks in the absence of food (Laurel et al. 2008). The compensatory growth mechanisms that Pacific cod larvae use to buffer early mismatches would also require basal rations prior to the match period. Alternatively, if spawning times of Pacific cod are variable enough year-to-year, a late emergence of larvae could potentially place larvae in ideal conditions. For example, a late spawning event coupled with a late, highly productive bloom in warm water could potentially put Pacific cod larvae in a "super match" situation, where growth and survival are maximized. An important area of future research will be to examine whether temperature – sea ice interactions, which appear to govern the timing and magnitude of prey production in the Bering Sea, also potentially affect the timing of spawning in Pacific cod, such that larvae are potentially placed in such ideal temperature/food conditions.

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Minimum viable population size for lake sturgeon (*Acipenser fulvescens*) using an individual-based model of demographics and genetics

Amy M. Schueller and Daniel B. Hayes

Abstract: Population viability analysis is a useful tool to explore the relationship between extinction risk and population size, but often does not include genetic factors. Our objectives were to determine minimum viable population size (MVP) for lake sturgeon (*Acipenser fulvescens*) and examine how inbreeding depression may affect MVP. Our individual-based model incorporated inbreeding depression in two ways: individuals with inbreeding coefficients above a threshold experienced inbreeding depression (threshold), and individuals experienced inbreeding depression at a rate related to their inbreeding coefficient (gradual). Three mechanisms relating inbreeding to fitness were explored (young-of-the-year (YOY) viability, post-YOY viability, number of progeny). The criterion we used to determine MVP was a 5% chance of extinction over 250 years. The estimated MVP without inbreeding effects was 80 individuals. For some scenarios incorporating inbreeding, MVP did not change, but for others, MVP was substantially higher, reaching values up to 1800. Results demonstrate that extinction risk and MVP can be influenced by both demographic stochasticity and inbreeding depression. This research should inform management by determining MVP and how inbreeding, which is expected to accrue in remnant populations because of generations of low abundance, may affect MVP.

Résumé : L'analyse de la viabilité d'une population est un outil utile pour explorer la relation qui existe entre le risque d'extinction et la taille d'une population, mais souvent elle n'inclut pas les facteurs génétiques. Nos objectifs sont de déterminer la taille minimale viable de la population (MVP) chez l'esturgeon jaune (*Acipenser fulvescens*) et l'effet potentiel de la dépression endogamique sur la MVP. Notre modèle basé sur l'individu incorpore la dépression endogamique de deux façons : les individus qui possèdent un coefficient d'endogamie au-dessus d'un certain seuil connaissent une dépression endogamique (seuil) et les individus connaissent une dépression endogamique à un taux proportionnel à leur coefficient d'endogamie (gradient). Nous avons exploré trois mécanismes qui relient l'endogamie à la fitness (la viabilité des jeunes de l'année (YOY), la viabilité après le stade YOY et le nombre de rejetons). Le critère que nous utilisons pour déterminer la MVP est une probabilité d'extinction de 5 % sur 250 ans. La MVP estimée sans les effets de l'endogamie est de 80 individus. Dans certains scénarios qui incorporent l'endogamie, MVP ne change pas, mais dans d'autres, la MVP est beaucoup plus élevée, pouvant atteindre des valeurs de l'ordre de 1800. Nos résultats démontrent que le risque d'extinction et la MVP peuvent être influencés par à la fois la stochasticité démographique et la dépression endogamique. Notre recherche devrait fournir des renseignements pour la gestion en déterminant la MVP et en montrant comment l'endogamie, qu'on s'attend à voir augmenter dans les populations résiduelles à cause de générations de faible abondance, peut affecter la MVP.

[Traduit par la Rédaction]

Introduction

Much debate has occurred over the past decades concerning the relative importance of demographic stochasticity (random births and deaths of individuals) and genetic stochasticity (random genetic changes due to genetic events such as inbreeding or drift; Shaffer 1981; Reed and Bryant 2000; Reed 2005) for the risk of extinction for small populations. Some have argued that demographic stochasticity is more important than genetics, particularly at small population sizes (Lande 1988; Harcourt 1991; Young 1991), based on the premise that if extinction occurs, no alleles are left to conserve. Several demographic factors, including edge effects, demographic stochasticity, and Allee effects, are thought to play larger roles in extinction processes for small population sizes (Lande 1988). However, others have demonstrated that inbreeding depression can lead to reduced population viability at small population sizes (i.e., increased

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A.M. Schueller[1,2] and D.B. Hayes. Michigan State University, Department of Fisheries and Wildlife, 13 Natural Resources Building, East Lansing, MI 48824, USA.

[1]Corresponding author (e-mail: amy.schueller@noaa.gov).
[2]Present address: National Marine Fisheries Service, Southeast Fisheries Science Center, NOAA Beaufort Laboratory, 101 Pivers Island Road, Beaufort, NC 28516, USA.

extinction risk). For example, Frankham (1995) concluded that the risk of extinction increased with inbreeding, and Keller and Waller (2002) found that many studies reported inbreeding depression can be detrimental to long-term persistence in the wild. Many argue that while inbreeding depression may not be the driving force towards extinction, inbreeding increases the risk of extinction (Mills and Smouse 1994). Therefore, a need exists to explore the relationship between demographics and genetics in regards to the long-term persistence of populations (Boyce 1992; Jager et al. 2000; Kirchner et al. 2006), particularly for long-lived species such as the lake sturgeon, *Acipenser fulvescens*. Focusing on long-lived species is especially important, since much of the research has focused on shorter-lived species with special assumptions (e.g., nonoverlapping generations), and longevity of a species likely provides a buffer against inbreeding, allele loss, and increased extinction risks for a population. For example, longevity and overlapping generations of the copper redhorse (*Moxostoma hubbsi*) have likely protected the species from reductions in genetic diversity and inbreeding with decreasing population sizes (Lippé et al. 2006).

Despite this need, few analyses have incorporated genetic factors into determinations of long-term population viability (Boyce 1992; Brook et al. 2002) or addressed how genetic stochasticity and the breeding system of an animal contribute to the minimum viable population size (hereafter, MVP) (Shaffer 1981). Some analyses have incorporated evolution and heritability of traits into modeling (Gomulkiewicz and Holt 1995; Gomulkiewicz and Houle 2009), but many have not included inbreeding and inbreeding depression. Recent studies of birds (Haig et al. 1993; O'Grady et al. 2006), plants (Burgman and Lamont 1992), mammals (O'Grady et al. 2006), invertebrates (Swindell and Bouzat 2006), and fishes (Brook et al. 2002) that examined demographic and genetic factors, specifically inbreeding depression, via population viability analysis have yielded inconsistent results (Mills and Smouse 1994; Brook et al. 2002). Fitness declines with increased inbreeding have been difficult to measure because of competing forces such as selection and drift and have been variable, ranging from no significant to significant changes, and sometimes fitness declines have been only temporary (Swindell and Bouzat 2006).

Population viability analysis (PVA) is a useful model-based tool that can take many forms, including statistical or mechanistic (Holmes et al. 2007), and allows for the exploration of the relationship between extinction risk and current population size (Shaffer 1981; Shaffer 1990; Boyce 1992). Many new improvements and modeling options have been developed for PVA (Holmes 2004; Staples et al. 2004; Holmes et al. 2007), but the end result is still an analysis of extinction or quasi-extinction risk (Holmes et al. 2007). Specifically, the PVA program called VORTEX has the capability to incorporate inbreeding depression (Caughley 1994). However, the majority of PVAs using VORTEX have only considered inbreeding depression on juvenile survival using the default values provided (Marshall and Edwards-Jones 1998). Results of PVA are often expressed as an MVP (Shaffer 1981). Generally, MVP is defined as a population of sufficient size to persist with a given probability over a given time frame, taking into account stochastic population processes (Shaffer 1981; Boyce 1992). MVPs are specific to each study or species and vary depending upon the species of interest, species' life history (e.g., population growth rate), time frame, factors considered (i.e., demographics, mutation, fitness), and tolerance of extinction risk by stakeholders for the species' management and conservation (Lindenmayer et al. 1993; Reed et al. 2003*b* and references therein; Traill et al. 2007). Both demographic and genetic stochasticity become increasingly important to a population's viability as population size decreases (Lindenmayer et al. 1993). Thus, both factors should be included in PVA, but modeling the influence of genetic changes on demography has proven to be difficult in many situations because translating negative genetic impacts into demographic impacts is not straightforward and genetic data are often unavailable (Beissinger and Westphal 1998; Kirchner et al. 2006).

One species where using PVA to explore MVP would be useful is the lake sturgeon. Lake sturgeon were once found throughout the Great Lakes basin in the United States and Canada (Birstein et al. 1997; Hay-Chmielewski and Whelan 1997; Smith and Baker 2005), but have been reduced to less than 1% of historic levels because of habitat degradation, overharvest, and fragmentation of spawning populations (Johnson et al. 1998; Auer 1999; Holey et al. 2000). Factors that contribute to the decline of lake sturgeon abundance also impede restoration where populations have been extirpated. Therefore, extensive discussions have occurred regarding management goals, objectives, actions, and research needs aimed at restoration and protection of the imperiled lake sturgeon. One of the research needs highlighted was determination of MVP (Auer 1996; Hay-Chmielewski and Whelan 1997).

Although lake sturgeon populations are much reduced in numbers, the genetic diversity of these populations has not been depressed yet, and there is no evidence of inbreeding (DeHaan et al. 2006; Welsh et al. 2008). Conserving the genetic integrity (defined here as both the retention of alleles and reduced inbreeding accrual; Schueller and Hayes 2010) of lake sturgeon should encompass maintaining the distribution of allelic diversity at genetic loci within a population, which is affected by inbreeding (Hay-Chmielewski and Whelan 1997; Keller and Waller 2002; McQuown et al. 2003). Inbreeding occurs when individuals who are closely related mate and most readily occurs in small populations where random mating can more readily result in breeding among related individuals (Hedrick and Kalinowski 2000; Brook et al. 2002; Keller and Waller 2002). Inbreeding typically results in decreased individual fitness relative to non-inbred offspring, termed inbreeding depression (Conner and Hartl 2004). Inbreeding depression can be manifested in any traits related to fitness (e.g., survival (viability), fecundity; Crnokrak and Roff 1999; Hedrick and Kalinowski 2000; Amos and Balmford 2001), but has been difficult to demonstrate empirically in wild populations (Newman and Pilson 1997).

Because of their longevity, it is difficult, if not impossible, to empirically determine the MVP for lake sturgeon and to fully document interactions between demographic and genetic processes leading to extinction. Therefore, we used a modeling approach to integrate both population dy-

namics and genetic information to inform management decisions and determine the relative contributions of demographics and genetics to extinction risk (Brook et al. 2002). The objective of our study was to determine the MVP for lake sturgeon, incorporating both demographic stochasticity and the potential impacts of inbreeding (Mills and Smouse 1994). This objective was addressed using an individual-based model (IBM) of demographic and genetic stochasticity. Of particular interest was modeled risk of extinction for different population sizes, but population-level genetic characteristics such as percentage of unique alleles lost and levels of inbreeding were also critical outcomes that we evaluated. The relative comparison of scenarios from this research will help to inform management about choices for rehabilitation of lake sturgeon populations within the Great Lakes basin. Finally, the IBM used here could be useful for many other species, as the model configuration is set up for versatility.

Materials and methods

Model description

An IBM was developed in SAS (SAS Institute Inc., Cary, North Carolina) that incorporated both demographic and genetic processes (Fig. 1; Schueller and Hayes 2010). The model mimicked the lake sturgeon's unique life-history traits, including delayed age at maturation (Bruch 1999; Billard and Lecointre 2001), sex-specific age at maturity, sex-specific spawning frequency (Auer 1999; DeHaan 2003), and mortality rates (see Schueller and Hayes 2010 for a complete model description; Baker 1980; Nichols et al. 2003; Table 1). Once an individual reached the minimum age for maturity, then the individual was assigned to mate each year or not based on sex-specific probabilities of mating. The lake sturgeon's polygamous mating system was mimicked, and the number of progeny surviving until the end of the summer from each mating pair was generated from empirical data (DeHaan 2003; Schueller 2009). Age-specific mortality rates were then applied to each individual whereby if a random number was below a specified level, the individual suffered mortality. Thus, demographic stochasticity was implicitly incorporated into the model because the birth and death of each individual was randomly determined. Additionally, overlapping generations are inherent in this model because all individuals are allowed to mate randomly with one another regardless of age. Populations were assumed closed (neither emigration nor immigration), which is supported by recent genetic research that suggests population differentiation (Welsh et al. 2008).

Each individual in the initial population, which consisted of post-young-of-the-year (YOY) males and females, had two unique alleles at a hypothetical locus, and each progeny produced also had a hypothetical locus, which was inherited as a Mendelian trait. No specific alleles were designated as deleterious. Retention of unique alleles was tracked using a technique called a gene drop analysis (MacCluer et al. 1986; Hedrick and Miller 1992; Haig et al. 1993). Individual inbreeding coefficients were calculated using pedigrees via path analysis using procedure INBREED in SAS (Wright 1922; SAS Institute Inc., Cary, North Carolina). All individuals at the start of the simulations were assumed to have zero inbreeding. The model was then expanded (see description below) from Schueller and Hayes (2010) to address the objective of this paper.

Fig. 1. Basic model structure for the individual-based simulation model used to explore minimum viable population size with and without inbreeding depression for lake sturgeon (modified from Schueller and Hayes 2010). YOY, young of the year.

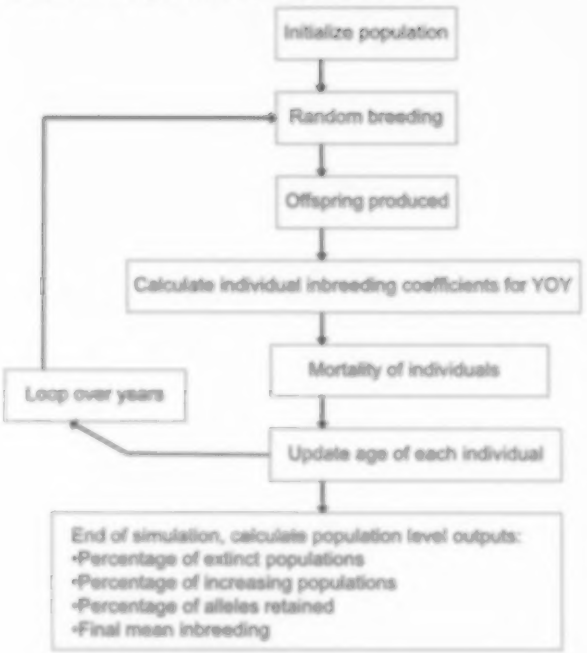


Table 1. Baseline parameter values to determine the minimum viable population size (modified from Schueller and Hayes 2010).

Parameter	Parameter value
Young-of-the-year mortality rate	0.47
Mortality rate	0.05
Age at first maturation for females	20
Probability of mating each year for females (once age at maturity has been reached)	0.33
Age at first maturation for males	15
Probability of mating each year for males (once age at maturity has been reached)	0.5

Minimum viable population size (MVP)

The MVP was considered the population size whereby the probability of extinction ($N = 0$) was approximately 5% over a 250-year timeframe (approximately 10 generations for lake sturgeon). A 5% risk of extinction is a common criterion for PVA (e.g., white-spotted char (*Salvelinus leucomaenis*), Morita and Yokota 2002; kaka (*Nestor meridionalis*), Leech et al. 2008; capercaillie (*Tetrao urogallus*), Marshall and Edwards-Jones 1998; Scott et al. 1995). A 250-year time frame has also been used in PVA for other long-lived species such as the bottlenose dolphin (*Tursiops truncatus*) (Thompson et al. 2000). MVP was determined for a scenario

with no inbreeding effects, which was then used for comparison with the scenarios that included inbreeding depression.

Few field studies have examined genetic impacts, including inbreeding and inbreeding depression, on population persistence outside of a captive environment (Ralls et al. 1988; Caughley 1994; Wang et al. 2002); this is particularly true when searching for field studies relating fitness changes to inbreeding coefficients calculated from a pedigree over multiple generations. The fitness effects that inbreeding has on lake sturgeon are currently unknown; therefore, we evaluated several plausible scenarios based on reports from other species. This was somewhat like a sensitivity analysis for inbreeding depression that determined the effect of fitness changes on long-term population persistence. The impacts of inbreeding depression on individual fitness were incorporated into the demographic model by creating demographic feedbacks for specified inbreeding scenarios. Two general types of scenarios were considered. In the first set of scenarios, reduced fitness was implemented by choosing an inbreeding level above which all individuals experienced reduced fitness. We termed this a "threshold" scenario and used values of 0.0625, 0.125, and 0.250. These coefficients represent levels of inbreeding that have been shown to reduce fitness in natural (Keller et al. 1994; Brown and Brown 1998), captive, and simulated populations of insects, birds, and mammals for the traits of interest in this study (Laikre and Ryman 1991; Reed et al. 2003*a*). In the next set of scenarios, reduced fitness was implemented as a linear decline in fitness with increasing individual inbreeding coefficients. We termed this a "gradual" scenario, meaning that different levels of individual inbreeding coefficients experienced different fitness declines, which were determined from a linear gradient. Given the observed range in inbreeding depression, across studies, species (DeRose and Roff 1999), and fitness components, of ~1%–19% for plants (Burgman and Lamont 1992), invertebrates (Moss et al. 2007), fish (Su et al. 1996; Wang et al. 2002; Thrower and Hard 2009), and mammals (Laikre et al. 1996; Lacy and Horner 1997; Laikre et al. 1997), we used slopes representative of the observed relationships between the inbreeding coefficient and the fitness component. Specific scenarios are described below. These inbreeding coefficients and scenarios (threshold and gradual) were chosen because they represent a range of plausible values and scenarios from past studies and because inbreeding has been found to affect different species with similar results (Crnokrak and Roff 1999; Reed et al. 2003*a*). Three traits affected by inbreeding depression were explored: YOY viability (i.e., survival), number of progeny, and post-YOY viability. Each of these traits has been shown to be affected by inbreeding depression across a variety of animal species, including fish (Hedrick and Miller 1992; DeRose and Roff 1999; Wang et al. 2002).

In total, nine inbreeding scenarios were explored for the threshold scenario. Three scenarios were explored for YOY viability using a threshold (0.0625, 0.125, and 0.250) whereby all individuals above the threshold did not survive. Hence, if the individual's inbreeding coefficient was above the specified threshold level, then the individual had a 0% chance of survival. This is the most extreme case of inbreeding depression possible for YOY viability because all individuals suffer mortality at the given thresholds. Three scenarios were explored for number of progeny using a threshold (as above) whereby all individuals above the threshold experienced a 10% reduction (on average per year) in the number of progeny produced. Three scenarios were explored for post-YOY viability using a threshold (as above) whereby individuals above the threshold had a reduced survival probability of 0.945 (which was a 10% increase in the post-YOY mortality rate to 0.055 from the baseline of 0.05).

In total, three inbreeding scenarios were explored for the gradual scenarios. One scenario was explored for YOY viability using gradual inbreeding whereby individuals were exposed to a reduced survival rate, which was related to their inbreeding coefficient by the relationship

$$y = 0.47f_x + 0.47$$

where y was the individual YOY mortality rate, f_x was the individual's inbreeding coefficient, and 0.47 was the baseline YOY mortality rate (Schueller and Hayes 2010). For every increase in the individual inbreeding coefficient of 0.10, the mortality rate increased by 10% from the baseline with a maximum mortality of 100%, which was the maximum for the threshold scenarios. A gradual change of 10% was used, as it was within the range reported in the literature for a meta-analysis (DeRose and Roff 1999), fishes (Su et al. 1996; Wang et al. 2002; Thrower and Hard 2009), mammals (Laikre et al. 1996; Lacy and Horner 1997; Laikre et al. 1997), and an invertebrate (Moss et al. 2007). For each of the gradual scenarios, the percent change of 10% was chosen as an intermediate value of observed inbreeding depression based on several studies. This value was chosen because we were aware of no specific relationship, based on empirical field data and inbreeding determined from a pedigree, available for lake sturgeon or for similar species. If inbreeding depression is determined to be greater or less than 10%, then extinction risks and associated MVPs may increase or decrease.

One scenario was explored for number of progeny using gradual inbreeding depression whereby individuals had a reduced number of progeny related to their inbreeding coefficient. The number of progeny was on average reduced 10% for each 0.10 increase in inbreeding coefficient. Finally, gradual inbreeding effects on post-YOY viability were explored where survival decreased by the relationship

$$w = 0.0208f_x + 0.05$$

where w was the individual post-YOY mortality rate, f_x was the individual's inbreeding coefficient, and 0.05 was the baseline post-YOY mortality rate (Table 1). The post-YOY mortality rate was able to increase until w reached a maximum increase in mortality of 10% at an inbreeding coefficient level of approximately 0.250, which was the maximum mortality increase for the post-YOY viability threshold scenarios. The slope that resulted from the relationship between the post-YOY mortality rate and inbreeding coefficient was 0.0208 and was solved for based on the criteria specified above. Because the gradual scenarios were limited by the minimum and maximum changes of the threshold scenarios, we were able to compare the threshold versus gradual scenarios directly.

Table 2. The minimum viable population size (MVP) for no inbreeding depression, threshold inbreeding depression where the threshold inbreeding level was provided (under Manifestation), and gradual inbreeding depression for several fitness components.

Inbreeding scenario	Manifestation	MVP	Final mean inbreeding coefficient	Alleles retained (%)	Diploid lethal equivalents
No inbreeding depression	NA	80	0.0913	7.63 (5.26–10.01)	NA
YOY viability	0.0625	125	0.0376	5.78 (3.69–7.86)	274.41
	0.125	100	0.0574	6.92 (4.65–9.19)	137.21
	0.25	80	0.0822	7.53 (5.17–9.89)	68.60
	Gradual	85	0.0902	7.28 (4.96–9.60)	3.94
Post-YOY viability	0.0625	80	0.0912	7.33 (5.00–9.66)	2.36
	0.125	80	0.0951	7.49 (5.13–9.84)	1.18
	0.25	80	0.0921	7.43 (5.08–9.77)	0.59
	Gradual	1800	0.0942	0.29 (0–0.78)[a]	0.62
No. of progeny	0.0625	150	0.0834	4.12 (2.34–5.90)	3.37
	0.125	150	0.0809	4.13 (2.35–5.90)	1.69
	0.25	150	0.0894	4.03 (2.27–5.79)	0.84
	Gradual	85	0.0877	7.28 (4.96–9.61)	4.61

Note: Data include the final mean inbreeding coefficient, the mean and approximate confidence intervals (±2 standard errors, SE) for the percentage of alleles retained at MVP, and the associated diploid lethal equivalents for each of the scenarios. For the percentage of alleles retained (approximate confidence intervals), simulations that went extinct were assigned a zero. YOY, young-of-the-year.

[a]Approximate confidence interval resulted in a negative value. The percentage of alleles retained cannot be negative; thus, the metric was represented as a zero.

For comparison, the maximum diploid lethal equivalents associated with each of the scenarios above were calculated using the methods in Morton et al. (1956) and O'Grady et al. (2006) (provided in Table 2). The method for two categories of data was used for threshold inbreeding, and the regression method was used for gradual inbreeding (Morton et al. 1956; O'Grady et al. 2006). For the threshold YOY viability scenarios, the rate for those individuals that were inbred (a zero survival rate) caused the equation to be undefined; thus, proxy diploid lethal equivalents were provided by using a survival rate of 0.0001. These proxies are large and represent situations in which YOY viability would be very low to nonexistent. This represents a worst-case scenario for a population. The diploid lethal equivalents for the remainder of the scenarios were all within the range of diploid lethal equivalents of two meta-analyses, which focus on plants and invertebrates (Armbruster and Reed 2005); birds and mammals (O'Grady et al. 2006); and for cultured coho salmon (*Oncorhynchus kisutch*) (Gallardo and Neira 2005).

Simulations

Simulations were run across a range of initial population sizes (10–2000 individuals, which represents many of the wild population sizes in the Great Lakes basin) to determine MVP based on the specified criterion. Each initial population size consisted of male and female post-YOY individuals. For each scenario, 500 simulations were run to provide estimates of the mean response and the variance across the factors explored. A yearly time step was used in all simulations. The percentage of extinct populations, average individual inbreeding coefficient in the final year, and the percentage of unique alleles retained were recorded for each simulation run of 250 years. The average individual inbreeding coefficient was the average inbreeding coefficient for progeny produced in the final year. The percentage of unique alleles was the average percentage of alleles retained from the initial population size (e.g., 100 individuals initially = 200 alleles), where extinct simulations were included as zero alleles retained. For the average percentage of alleles retained, approximate confidence intervals were calculated as

$$(\text{estimate} \pm 2\,\text{SE}) \times 100$$

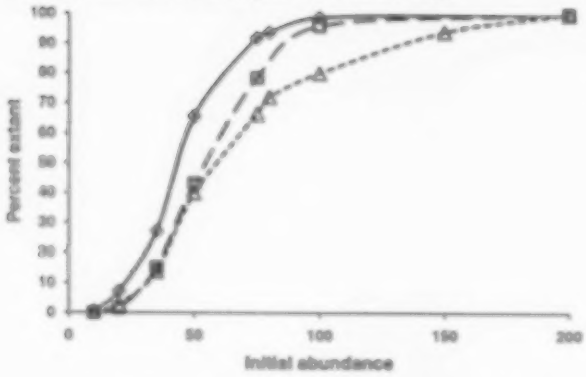
where $\text{SE} = \sqrt{\frac{pq}{n}}$, p = proportion of alleles retained, $q = 1 - p$, and $n = 500$. Approximate confidence intervals for the percentage of extant populations were also calculated for each scenario using proportions, but the error bars were not graphed because the bars fell within the markers on the graphs. Simulations were assumed to have current environmental and demographic conditions, which remained constant into the future, and differences in fitness between individuals related to life-history variation were assumed to be zero.

Results

The MVP for our baseline scenario, which included demographic stochasticity but no inbreeding effects, was approximately 80 individuals (male and female post-YOY individuals). The risk of extinction increased rapidly for smaller populations; initial populations of 40 had an approximately 50% extinction rate, and populations of 15 or fewer went extinct in virtually all cases (Fig. 2) for simulations starting at the MVP of 80. The final inbreeding coefficient averaged 0.0913, and the percentage of alleles retained was 7.63% (95% confidence interval, CI = 5.26 to 10.01; Table 2).

For many of the scenarios explored, the MVP was not substantially increased by inbreeding depression (Table 2), as the MVPs were the same as or close to the MVP with no

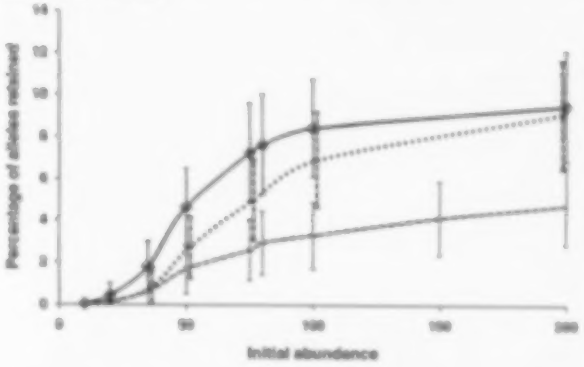
Fig. 2. The percentage of populations that remained extant for a range of initial population sizes (called a persistence curve) for scenarios with no inbreeding (diamond with solid line), with threshold inbreeding depression as reduced number of progeny at the threshold 0.125 (triangle with dashed line), and with threshold inbreeding depression as decreased young-of-the-year (YOY) viability at the threshold 0.125 (square with dashed line).



inbreeding depression. In particular, MVP was 80 for decreased post-YOY viability for all thresholds and was 85 when inbreeding depression occurred as gradual impacts on YOY viability and number of progeny. However, MVP increased when inbreeding depression was incorporated as a reduction in the number of progeny or YOY viability based on inbreeding thresholds and when post-YOY viability gradually decreased as a function of inbreeding. MVP was 150 with a reduced number of progeny for all thresholds. MVPs ranged from 80 to 125, with lower thresholds leading to increased MVP for YOY viability. The only scenario where MVP was vastly different was with gradual inbreeding depression with a decrease in post-YOY viability. Under this scenario, MVP was approximately 1800.

When inbreeding depression resulted in an MVP different from 80, the persistence curve (the curve of percentage of extant populations versus initial population size) changed when compared with the persistence curve with no inbreeding depression. MVP is a point on the persistence curve with a given extinction risk, and as the initial population size increases or decreases, the risks of extinction change differently for the different scenarios (Fig. 2). When the MVP changed, the persistence curves maintained high risk at small population sizes and lower risk at large populations sizes. However, at intermediate population sizes, the risk of extinction changed at different rates. For example, the rate at which the persistence curve reached 100% decreased as the initial population size was increased with threshold inbreeding depression as reduced number of progeny (Fig. 2). Some of the curves maintained a similar shape but simply shifted. For example, the persistence curve shifted causing an increase in the MVP for threshold inbreeding depression on YOY viability (Fig. 2). The persistence curves were very similar when compared with the persistence curve with no inbreeding depression when the MVP was similar (e.g., gradual inbreeding depression for number of progeny). Therefore, for clarity, not all persistence curves were graphed.

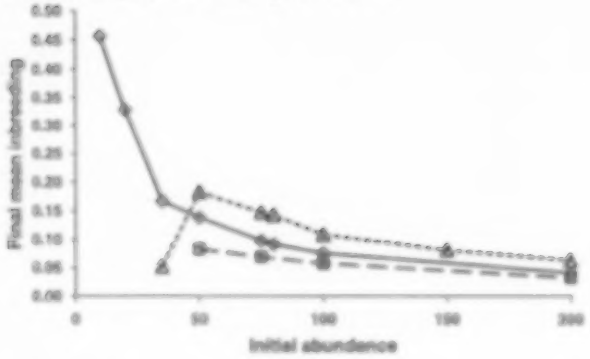
Fig. 3. Percentage of alleles retained across a range of initial population sizes under the scenarios of no inbreeding (diamond with solid line), threshold inbreeding depression as reduced number of progeny at the threshold 0.125 (dashed line), and threshold inbreeding depression as decreased young-of-the-year (YOY) viability at the threshold 0.125 (dotted line). The percentage of alleles retained represents all simulations, including populations with no survivors. Approximate confidence intervals (±2 standard errors, SE) for each scenario were represented in the same line type for each scenario. Simulations with no survivors were considered to have no alleles retained. Some of the error bars are offset slightly for differentiation.



When inbreeding depression increased the MVP, the percentage of alleles retained decreased, but the impact on the final mean inbreeding coefficient depended upon the scenario (Table 2). The percentage of alleles retained decreased slightly for threshold inbreeding depression on YOY viability and decreased greatly (approximately halved) for threshold inbreeding depression as reduced number of progeny when compared with the scenario without inbreeding (Table 2; Fig. 3). The final mean inbreeding level increased for threshold inbreeding depression as reduced number of progeny and the final mean inbreeding level decreased for threshold inbreeding depression as decreased YOY viability when compared with the no inbreeding scenario (Fig. 4). The final mean inbreeding coefficient and the percentage of alleles retained both increased with increasing thresholds for the inbreeding depression scenarios for YOY viability (Table 2). The final mean inbreeding coefficient was similar to the no inbreeding scenario, but the percentage of alleles retained was greatly reduced when compared with the no inbreeding scenario for inbreeding depression via gradual post-YOY viability.

Finally, in scenarios where MVP was similar to the no inbreeding depression simulations, the percentage of alleles retained and the final mean inbreeding coefficient were also similar (Table 2). The final mean inbreeding coefficient and the percentage of alleles retained for all of the threshold scenarios for post-YOY viability and for the gradual YOY viability scenarios were similar to the scenario with no inbreeding effects. The final mean inbreeding coefficient was slightly less, but the percentage of alleles retained was similar for the gradually reduced number of progeny scenario when compared with the no inbreeding scenario. Because of the similarities in results between scenarios, not all scenarios were graphed.

Fig. 4. Final mean inbreeding across a range of initial population sizes under the scenarios of no inbreeding (diamond with solid line), threshold inbreeding depression as reduced number of progeny at the threshold 0.125 (triangle with dashed line), and threshold inbreeding depression as decreased young-of-the-year (YOY) viability at the threshold 0.125 (square with dashed line). Final inbreeding coefficients represent only extant populations at the end of the 250-year simulation duration; populations with no survivors were not assigned an inbreeding coefficient.



Discussion

Traditionally, PVA models, which explore MVP, focus on demographic and environmental stochasticity. Often, genetic components are not incorporated, but can have an important impact on a population's persistence (Boyce 1992; Jager et al. 2000; Kirchner et al. 2006). As such, the IBM developed could be useful for many species to determine MVPs and relative extinction risks, which incorporate both demographic stochasticity and inbreeding effects. Incorporating both demographics and genetics to determine the MVP may be important but has not been widely explored for fishes (Boyce 1992; Reed et al. 2003*b*; Traill et al. 2007). For example, only one fish species out of nine included in the large meta-analysis of MVP by Traill et al. (2007) included inbreeding effects. As such, our findings on the potential influence of inbreeding depression on a long-lived fish species are unique.

The probability of extinction can never truly be zero for any wild population (Shaffer 1990), but management can strive to minimize the risk of extinction by determining MVP and maintaining populations above that level. Based on our model, we estimated that the MVP for lake sturgeon populations in the Great Lakes basin was 80–150 post-YOY male and female individuals with approximately a 5% risk of extinction over 250 years. This population size is much smaller than the 1188 spawning females estimated to be necessary for persistence of lake sturgeon in the stage-structured assessment by Vélez-Espino and Koops (2009); however, their analysis used a much more stringent criterion for persistence of 99% extant over 40 generations. Few MVPs have been determined for other fish species; only nine of 287 MVPs were for fish species in the original data set compiled for the meta-analysis by Traill et al. (2007), and only one of 102 for a study by Reed et al. (2003*b*). Those MVPs that have been estimated for other fish species are generally larger than for the MVP estimated for lake sturgeon, which could be related to different life-history strategies between species or different time frames or criteria used to estimate MVP. For example, the MVP for white-spotted charr was estimated to be 250 with a 5% risk of extinction over 100 years (Morita and Yokota 2002), and Traill et al. (2007) predicted a standardized MVP using a general linear mixed effects model for eight fish species to range from approximately 200 000 to 2 million. The MVP we estimated for lake sturgeon was, however, comparable to MVPs for other long-lived vertebrate species. The MVP was found to be 125 grizzly bears (*Ursos arctus horribilis*) for 100 years (Suchy et al. 1985) and about 100 Japanese black bears (*Ursus thibetanus japonicus*) for 100 years with a 5% risk of extinction (Horino and Miura 2000). A population of approximately 120 Asian elephants (*Elephas maximus*) was determined to be the MVP with about a 5% risk of extinction over 1000 years (Armbruster et al. 1999).

Little empirical evidence is available relating levels of inbreeding to MVP; therefore, modeling plausible scenarios is a useful strategy to explore possible effects. Theory suggests inbreeding depression increases MVP because individuals in an inbred population have lower fitness than individuals in a non-inbred population of the same size (Amos and Balmford 2001). For example, increased levels of inbreeding depression in red cockaded woodpeckers (*Picoides borealis*) resulted in increased risks of extinction based on analyses using the population viability analysis software VORTEX (Haig et al. 1993). Similarly, the MVP using VORTEX for the Atlantic forest spiny rat (*Trinomys eliasi*) was 10 times higher when inbreeding was included than when inbreeding was not (Brito and Figueiredo 2003). Finally, extinction risks were sensitive to reduced juvenile survival of inbred long-furred woolly mouse opossum (*Micoureus paraguayanus*) (Brito and Fonseca 2006). When genetic factors have been incorporated into estimates of MVP in the past, they have often been incorporated using the program VORTEX (Caughley 1994). Although inbreeding depression can be incorporated in VORTEX for a number of fitness components, the majority of analyses have only considered juvenile survival (Marshall and Edwards-Jones 1998). Further, many analyses have used only the default values provided in VORTEX because of the lack of empirical measurements of inbreeding depression (Marshall and Edwards-Jones 1998). In contrast, a few studies have explored a higher level of inbreeding depression using VORTEX to look at extinction risks (O'Grady et al. 2006) and management scenarios (Zachos et al. 2009).

One important difference between our study and others is that we used an IBM, and the individual inbreeding coefficients were calculated through path analysis. The inbreeding coefficient (f_x) calculated in our study via path analysis is a direct probability of inbreeding or of alleles being identical by descent, in contrast with studies using effective population size or other proxies (Su et al. 1996). This allowed for those individuals that were the most inbred to be subjected to reduced fitness instead of the population as a whole being subjected to reduced fitness.

Some inbreeding depression scenarios explored in this analysis resulted in little or no change to the MVP when compared with the scenario without inbreeding depression. With inbreeding depression under the threshold scenario,

the MVP was the same as the MVP with no inbreeding depression for post-YOY viability for all thresholds. With gradual inbreeding depression for both YOY viability and number of progeny, the MVP increased only slightly to 85. Similar results were found in other studies. For example, increased probabilities of extinction were not predicted for the plant species *Banksia cuneata* with increased levels of inbreeding (Burgman and Lamont 1992). Inbreeding depression was thought not to increase extinction risks in Burgman and Lamont's model (1992) because of the random effects of environmental stochasticity.

Some inbreeding depression scenarios did result in changes to the predicted MVP. With threshold inbreeding depression on number of progeny, the MVP was 150, nearly twice the MVP with no inbreeding depression. This result is similar to other findings where small increases in inbreeding depression for survival and fecundity parameters increased the risk of extinction for populations compared with simulations without inbreeding (Mills and Smouse 1994). Also, the probability of extinction increased for 20 species when inbreeding depression on juvenile mortality was included in simulation models (Brook et al. 2002). For the bird capercaillie (*Tetrao urogallus*), the MVP with a 5% extinction risk was about 150 individuals with inbreeding depression manifested for juvenile survival in VORTEX (Grimm and Storch 2000). Finally, the MVP including inbreeding depression on pup mortality was 400 individuals for wolves (*Canis lupus*) in Scandinavia (Nilsson 2004). Thus, it appears that the influence of inbreeding depression on extinction risk depends strongly on which fitness components show a manifestation of inbreeding depression.

We found that gradual inbreeding depression via post-YOY viability resulted in an MVP of approximately 1800. Given the current lake sturgeon population sizes in the Great Lakes basin, this scenario seems implausible because most populations are below 1800 and have not reached extinction during the recent generations at low abundance (Hay-Chmielewski and Whelan 1997). We hypothesize that this large MVP in this scenario is due to the compounding effects of increased mortality over time on the population. In a sensitivity analysis of this lake sturgeon population model (Schueller and Hayes 2010), we found that extinction rates and abundance were most sensitive to post-YOY mortality. Sensitivity to post-YOY survival has been found for other long-lived species (Boyce 1992), and thus, this model result makes sense given that even small increases in the post-YOY mortality rate resulted in large increases in the probability of extinction.

For the scenarios that incorporated inbreeding depression gradually, the estimated MVP was likely dependent on the slope of the fitness function. Because the model outputs were hypersensitive to changes in the population parameters related to survival (Schueller and Hayes 2010), the MVP for these scenarios will likely change with changes in survival. For example, the model was most sensitive to post-YOY mortality, and gradual inbreeding depression on post-YOY mortality resulted in the largest MVP. This example illustrates that the slope chosen for these scenarios can greatly influence the outcome. Therefore, a critical need exists to obtain better empirical estimates of inbreeding depression on sensitive population parameters.

With threshold inbreeding on YOY viability, the MVP increased with decreasing thresholds . Although inbreeding depression reduces fitness, mortality of inbred individuals prior to reproduction leads them to not contribute to the next generation. Thus, these inbred individuals are "purged" from the population. This process can lead to fitness levels being maintained or sometimes increased in future generations (Hedrick and Kalinowski 2000; Crnokrak and Barrett 2002; Keller and Waller 2002). Thus purging can lead to situations where deleterious alleles are lost and inbreeding depression is reduced in future generations (Leberg and Firmin 2008). We found that as the threshold increased, the final mean inbreeding coefficient increased, as did the percentage of alleles retained. This is an example of purging where individuals with inbreeding coefficients above the threshold experience mortality as YOY. This results in the removal of those individuals with higher inbreeding coefficients, leading to lower mean inbreeding coefficients than what would be expected without purging (Crnokrak and Barrett 2002). Inbreeding depression was likely reduced by purging for *Drosophila melanogaster* with small population sizes (Swindell and Bouzat 2006), and purging appears to be important to fitness in plants, mammals, mollusks, and insects (Crnokrak and Barrett 2002).

Demographic stochasticity often dominated the extinction process for lake sturgeon because the MVP was often the same or only slightly larger with inbreeding depression. For example, with the addition of inbreeding depression for post-YOY viability under threshold scenarios, the MVP was still 80. However, MVP was influenced by inbreeding depression, depending upon the scenario. For example, with inbreeding under the threshold scenario, the MVP for the number of progeny scenarios was 150 or nearly twice the MVP with no inbreeding effects. Much discussion has occurred over whether demographic stochasticity or genetic stochasticity is more important or has a greater influence on long-term persistence (Lande 1988; Frankham 1995; Spielman et al. 2004). Based on the scenarios in our study, whether demographic or genetic stochasticity dominated the determination of MVP and extinction risk was dependent upon whether inbreeding depression was expressed as threshold or gradual and whether inbreeding depression affected viability or the number of progeny. Thus, our study does not provide a clear picture of whether demographic or genetic stochasticity dominated extinction risk but supports the idea that both processes are involved. Thus, efforts should be made to understand and document how genetic and demographic factors jointly affect MVP.

Often, measuring inbreeding depression in wild populations and habitats is difficult because of the many environmental factors that impact populations and individuals (Pimm et al. 1988; Reed and Bryant 2000). One factor we did not include was environmental stochasticity. When inbreeding depression and environmental stochasticity act simultaneously, an inbreeding vortex can occur whereby populations reach extinction at a quicker rate (Tanaka 2000). Thus, the incorporation of environmental stochasticity to this model could increase rates of extinction for smaller population sizes via the inbreeding vortex. Therefore, larger populations may need to be maintained for threatened and endangered species to protect against an in-

breeding vortex resulting from demographic stochasticity, the plausible effects of inbreeding depression, and environmental stochasticity. Environmental stochasticity was not included in this model because of the longevity of lake sturgeon, and because year to year variation in population parameters would most likely not be large enough to cause differences in population persistence over the long run (Benton et al. 1995). Moreover, Melbourne and Hastings (2008) showed that environmental stochasticity was less of a factor for extinction risk if demographic heterogeneity (random births and deaths) and stochasticity in sex ratio were included in the model. This was especially true when population sizes were small or declining (Melbourne and Hastings 2008). Our analysis explored smaller population sizes, our model had random births and deaths, and an emergent property of our model was a stochastic sex ratio; thus, including environmental stochasticity would potentially not have increased extinction risk substantially. Finally, no long-term quantitative estimates of stochastic variability are available for critical fitness components of lake sturgeon.

As a final caveat, as shown in Schueller and Hayes (2010), the outputs of the model were hypersensitive to changes in the population parameters (e.g., YOY mortality and post-YOY mortality). Thus, if the baseline parameter values vary, then the MVP as estimated above would likely change, potentially significantly. For example, if either of the mortality rates are increased or decreased, the MVP would likely increase or decrease, respectively. Thus, the estimate of MVP is dependent upon the baseline parameters used. Additionally, the scenarios explored are likely not mutually exclusive in nature, and thus these estimates could be considered as lower bound on MVP. Given the uncertainty in baseline parameter values, we feel our estimates of MVP should not be treated as definitive values. Rather, these values are useful for management purposes as guidance until more precise inputs can be developed.

Recommendations

Because this work examines the plausible role that inbreeding depression may play in the extinction process, this work should be used to guide experimental research looking to measure the effects of inbreeding depression. Thus, if inbreeding depression is manifesting itself in lake sturgeon or other long-lived species, inbreeding depression should be most readily measured as a reduced number of progeny. Also, if inbreeding depression is resulting in purging of a population, then YOY viability should be investigated.

Given that in some inbreeding depression scenarios the MVP was increased, we recommend increasing the MVP to account for the possibility of inbreeding depression for other threatened and endangered species to overcome both demographic stochasticity and maintain genetic integrity. Because lake sturgeon are long-lived and because of their life-history characteristics (e.g., iteroparous, overlapping generations), a much lower MVP may have resulted when compared with most other species. Shorter life spans (e.g., those of insects) most likely require populations of greater size to maintain population persistence through time (Pimm et al. 1988; Boyce 1992). Therefore, other threatened and endangered species that do not have long life spans should have their populations maintained at much higher levels if using the given time frame of 250 years, and those species that are long-lived, with similar population and life-history parameters to the baseline parameters used here, might at least need populations maintained above 80–150 individuals. Given the uncertainty in these parameter estimates, larger population sizes may still be required for persistence.

Lake sturgeon long-term dynamics and persistence are dependent upon maintaining populations that are above the MVP while also maintaining genetic integrity. Thus, we recommend that populations should be maintained above 80 to 150 individuals, for the given baseline parameters explored. However, given the uncertainty in baseline parameter values, our estimates of MVP should not be treated as absolute numbers, but as values useful for management purposes, which provide guidance until more precise inputs can be developed. These recommendations should help to foster the protection of sustainable lake sturgeon populations for the future by minimizing extinction risks due to both demographic stochasticity and inbreeding depression. Also, estimation of MVP should help lake sturgeon managers prioritize populations for future rehabilitation efforts, given extinction risks. Given populations are below the MVP level, management efforts such as some form of supplementation may be necessary to overcome demographic stochasticity and maintain persistence.

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Urban stressors alter the trophic basis of secondary production in an agricultural stream

Björn Gücker, Mario Brauns, Angelo G. Solimini, Maren Voss, Norbert Walz, and Martin T. Pusch

Abstract: We compared the invertebrate production and stable isotope signatures of key ecosystem compartments of urban sites subjected to the input of tertiary-treated wastewater with those of upstream sites in an agricultural lowland stream. We detected a significant shift in the trophic basis of invertebrate production from upstream, natural and agricultural resources, to urban resources, i.e., wastewater-derived organic matter as well as autochthonous primary production based on wastewater-derived nutrients. Invertebrate production was higher at urban sites than at agricultural sites. However, the median contribution of the most important secondary producer, the shredder *Gammarus roeseli*, to total invertebrate production was lower at urban sites (9%) than at agricultural sites (61%). The low production of *G. roeseli* at urban sites was associated with the absence of allochthonous course particulate organic matter (CPOM) habitats, rather than the loss of CPOM as a food resource. Our results suggest that contemporary urban stressors in developed countries affect secondary producers less severely than historically recorded, but still profoundly change the matter fluxes and ecosystem functioning of running waters. Restoration of the native riparian vegetation, channel naturalization, and adequate dilution of tertiary-treated wastewater may partially mitigate adverse effects on invertebrate communities and their secondary production.

Résumé : Nous comparons la production d'invertébrés et les signatures d'isotopes stables de compartiments clés de l'écosystème dans des sites urbains soumis à l'apport d'eaux usées de traitement tertiaire par comparaison à des sites d'amont dans un cours d'eau agricole de plaine. Nous détectons un changement significatif dans la base trophique de la production d'invertébrés, à partir des ressources naturelles et agricoles en amont vers des ressources urbaines, c'est-à-dire de la matière organique dérivée des eaux usées, ainsi qu'une production primaire autochtone basée sur des nutriments provenant des eaux usées. La production d'invertébrés est plus élevée dans les sites urbains que dans les sites agricoles. Cependant, la contribution médiane du producteur secondaire le plus important, le déchiqueteur *Gammarus roeseli*, à la production totale des invertébrés est plus basse aux sites urbains (9 %) qu'aux sites agricoles (61 %). La faible production de *G. roeseli* aux sites urbains est associée à l'absence d'habitats à matière organique particulaire grossière (CPOM) allochtone, plutôt qu'à la perte de CPOM comme ressource alimentaire. Nos résultats laissent croire que les facteurs urbains actuels de stress dans les pays développés affectent les producteurs secondaires moins sévèrement que signalé dans le passé, mais qu'ils modifient néanmoins profondément les flux de matière et le fonctionnement de l'écosystème dans les eaux courantes. La restauration de la végétation riveraine indigène, la naturalisation du chenal et la dilution adéquate des eaux usées après un traitement tertiaire peuvent en partie réduire les effets négatifs sur les communautés d'invertébrés et leur production secondaire.

[Traduit par la Rédaction]

Introduction

Streams and rivers are of outstanding importance to human culture, welfare, and development, but are concomitantly highly vulnerable to urbanization and associated water use (Malmqvist and Rundle 2002). Dramatic deterioration of the water quality of urban streams owing to the discharge of crudely treated organically rich wastewater has been documented for more than a century (Hynes 1974; Spitta 1900; Welch and Lindell 1992). With the widespread inception of modern wastewater treatment in developed countries, the dominant impacts of urbanization may have shifted from organic pollution to a more diverse array of stressors. Indeed, contemporary urban streams consistently exhibit incised and straightened channels, reduced in-channel complexity, flashier hydrographs, and increased biological oxygen demand, as well as increased concentrations of inorganic nutrients, suspended solids, hydrocarbons and metals,

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B. Gücker.[1] Federal University of São João del Rei, Department of Biosystems Engineering, Campus Tancredo Neves, Avenida Visconde do Rio Preto, Colônia do Bengo, 36301-360, São João del-Rei, Minas Gerais, Brazil.
M. Brauns, N. Walz, and M. Pusch. Leibniz Institute of Freshwater Ecology and Inland Fisheries, Department Limnology of Shallow Lakes and Lowland Rivers, Müggelseedamm 301, 12573 Berlin, Germany.
A.G. Solimini. Department of Public Health and Infectious Diseases, Sapienza University of Rome, Piazzale Aldo Moro 5, 00161 Rome, Italy.
M. Voss. Leibniz Institute for Baltic Sea Research, Seestrasse 15, 18119 Rostock, Germany.

[1]Corresponding author (e-mail: guecker@ufsj.edu.br).

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thereby still resulting in impoverished biological communities dominated by pollution- and disturbance-tolerant species (see reviews by Paul and Meyer 2001; Walsh et al. 2005).

While adverse effects of urban stressors on stream biodiversity are well documented (Paul and Meyer 2001; Allan 2004), little is known about their effects on stream ecosystem functioning. Therefore, the study of ecosystem metabolism, nutrient and organic carbon (OC) processing, and secondary production has been identified as a major research need in urban stream ecology (Paul and Meyer 2001; Walsh et al. 2005). Studies comparing ecosystem functioning among streams draining urban and natural, as well as urban and agricultural catchments indicate that urban stressors increase stream metabolism (Sloane-Richey et al. 1981; Paul 1999; Gücker et al. 2006), decomposition of organic matter (Collier and Winterbourn 1986; Paul et al. 2006; Spänhoff et al. 2007), secondary production (Shieh et al. 2002; deBruyn et al. 2003), and area-specific nutrient uptake, but decrease flux-specific nutrient uptake, i.e., the inverse of the nutrient uptake length (Gücker and Pusch 2006; Mulholland et al. 2008).

Modern wastewater treatment typically comprises three stages, i.e., primary, secondary, and tertiary treatment. In brief, large particulate organic and mineral material is removed from the wastewater in primary treatment. In secondary treatment, bacteria and protists degrade organic matter in activated sludge or fixed film systems. As a rather recent innovation, inorganic nutrients are removed from the wastewater in tertiary treatment by microbial nitrification/denitrification, biological phosphorus (P) removal, or chemical P precipitation. The type of wastewater treatment and the ratio of wastewater to total stream discharge seem to be critical factors determining urban impacts on stream ecosystem functioning. For example, high secondary production at an urban site of the fifth-order river, La Poudre, in Colorado, USA, was associated with low predation pressure and high inorganic P and N availability, owing to the discharge of a secondary wastewater treatment plant (WWTP) (Shieh et al. 2002). In contrast, two of the first studies to quantify human impacts on ecosystem functioning of a large river (St. Lawrence River, Quebec, Canada) demonstrated that high secondary production at urban sites was a result of the assimilation of particulate organic matter (POM) derived from primary-treated wastewater (deBruyn and Rasmussen 2002; deBruyn et al. 2003). Similarly, increases in secondary production owing to the discharge of a rural secondary WWTP into an Austrian third-order forest stream were attributed to the assimilation of P-enriched wastewater-derived POM (Singer and Battin 2007). However, tertiary wastewater treatment has become common in developed countries. For example, nearly 100% of the German population was connected to wastewater treatment facilities in 2005 and tertiary treatment was applied to the wastewater of 94% of the population (Verbände der Wasserwirtschaft 2005). Surprisingly, there is, to our knowledge, no published work available regarding the effects of tertiary-treated wastewater on stream secondary production.

In the present study, we investigated the effects of contemporary urban stressors on the secondary production of an agricultural lowland stream near Berlin, Germany, focusing on impacts of substantial tertiary-treated wastewater discharge and riparian clearcutting. Thus, we studied the effects of these urban stressors on a stream already impacted by agricultural land use and not those on pristine streams. This approach is of great practical relevance and interest, because urban centres are frequently surrounded by an extensive gradient of urbanization and agriculture, and pristine streams are rarely exposed to urban stressors. We hypothesized that the effects of these common urban stressors on secondary production would be less dramatic compared with the historically prevailing effects of crudely treated wastewater discharge. Further, we hypothesized that these stressors would still affect the availability and the nutritional quality of food resources, e.g., through the effects of eutrophication on periphyton and macrophyte growth or the reduction of riparian coarse particulate organic matter (CPOM) inputs, which would subsequently affect stream secondary production. Based on the stable isotope composition (Peterson 1999) and nutrient stoichiometry (Sterner and Elser 2002) of secondary producers and their food resources, we examined whether changes in stream secondary production were linked to alterations in the availability, origin, and elemental stoichiometry of food resources.

Materials and methods

Study sites

Our study was performed at the Erpe, a tributary to the River Spree in Berlin, Germany. Three sampling sites were established in both a third-order section of the upper (upstream of 52°29′16″N and 13°38′46″E, 207 km^2 catchment size) and the lower catchment (downstream of 52°29′00″N and 13°38′28″E, 211 km^2 catchment size including the upper catchment) of the Erpe. Sampling sites were about 0.5 km away from each other and the first sampling site in the lower catchment was about 200 m downstream of the wastewater outfall. There are no tributaries to the Erpe in the studied stream section. The upper catchment has a population density of 127 inhabitants·km^{-2} and is, according to the CORINE map (European Environmental Agency 1997), dominated by agricultural land use on 74.5% of the catchment area. Urban area and forest comprise 13.6% and 11.7% of the catchment area, respectively. In contrast, the lower catchment has a population density of 1575 inhabitants·km^{-2} and is dominated by urban area (50.1%), followed by agricultural area (24.9%) and forest (24.9%). Moreover, the lower Erpe receives more than 200 000 population equivalents of tertiary-treated wastewater, roughly tripling the stream's discharge from 211 (107–1660) L·s^{-1} in the upper catchment to 578 (462–2215) L·s^{-1} in the lower catchment (medians and ranges for 2003 from daily measurements). As a result, the lower Erpe remains eutrophic up to its mouth in Berlin–Köpenick with stream water nutrient concentrations of 5–12 mg NO_3-N·L^{-1}, 0.06–0.16 mg NH_4-N·L^{-1}, and 0.07–0.40 mg SRP·L^{-1} (D. Olbrich, Municipality of Berlin, Berlin, Germany, personal communication 2004). Henceforth, the sampling sites in the upper catchment will be referred to as "agricultural sites" and those in the lower catchment will be referred to as "urban sites". Mean stream channel depths and widths were 0.44 ± 0.06 m (mean ± SD of the three sites) and 3.3 ± 0.4 m, respectively,

at agricultural sites and 0.76 ± 0.15 m and 4.9 ± 0.3 m at urban sites. Agricultural sites of the Erpe receive considerable amounts of nutrients through diffuse sources (Table 1). Owing to the discharge of tertiary-treated wastewater and associated eutrophication, urban sites exhibit higher rates of gross primary production and ecosystem respiration than agricultural sites (Gücker et al. 2006). The Erpe has riparian oak and alder forests, but is mainly an open-canopy stream. All investigated sites had riparian forest on the left stream bank and pasture without any riparian trees or shrubs on the right bank. However, at agricultural sites, the riparian forest was in immediate proximity to the left bank while riparian clearcutting at urban sites prevented the growth of riparian trees at a distance between 2 m and 4 m from the left stream bank. Autotrophic production in the Erpe is dominated by submerged macrophytes growing from May to September (Gücker et al. 2006), mainly *Sparganium emersum* Rehmann and *Potamogeton pectinatus* L., but also *Glyceria maxima* [Hartman] Holmberg, and *Elodea canadensis* Michx. The biomass of submerged macrophytes reached levels 7- to 9-fold higher in urban sites than in agricultural sites (Gücker et al. 2006). Large sections of the Erpe, including the studied sites, are incised and straightened and have predominantly clogged and anaerobic fine-sand siliceous sediments with high organic carbon contents (Table 1).

Table 1. Physical and chemical characteristics of the Erpe.

	Agricultural sites	Urban sites	TTWW
Physical variables			
Discharge ($L \cdot s^{-1}$)*	188±32	548±54	346±40
Current velocity ($cm \cdot s^{-1}$)*	14.1±1.4	16.9±1.9	NA
Water temperature (°C)*	11.8±4.9	14.4±3.5	16.2±2.9
Conductivity ($\mu S \cdot cm^{-1}$)*	783±56	1196±78	1398±136
Sediments			
Median grain size (mm)	0.24±0.06	0.22±0.04	NA
AFDM ($g \cdot m^{-2}$)*	2258±249	3091±450	NA
Water chemistry			
DO ($mg \cdot L^{-1}$)*	9.7±1.7	9.3±1.1	9.0±1.1
pH*	7.9±0.2	7.6±1.3	7.5±0.2
NO_3-N ($mg \cdot L^{-1}$)*	2.4±1.9	10.1±3.0	13.7±4.0
NH_4-N ($\mu g \cdot L^{-1}$)*	85±57	104±63	112±75
SRP ($\mu g \cdot L^{-1}$)*	49±22	338±91	480±146
TP ($\mu g \cdot L^{-1}$)*	148±44	553±113	749±175
DOC ($mg \cdot L^{-1}$)*	6.1±1.2	9.7±1.1	11.4±1.7
POC ($mg \cdot L^{-1}$)*	1.9±0.3	2.3±0.4	2.5±0.4

Note: Data are annual means ± 1 SD for the year 2003 from biweekly midday samplings. TTWW, tertiary-treated wastewater; NA, data not available. Significant differences between urban and agricultural sites are indicated beside the respective variable; *, $P < 0.05$, according to paired Wilcoxon tests.

Invertebrate sampling

To estimate invertebrate secondary production, invertebrates were sampled in five sampling campaigns in March 2002, June 2002, December 2002, March 2003, and May 2003. A Surber sampler (sampled area 0.09 m^2, 200 µm mesh; Hydrobios, Kiel-Holtenau, Germany) was used to collect benthic invertebrates from the surface sediments, including those on macrophytes and allochthonous CPOM on the sampled sediment surface, from each of the six sampling sites in each of the five sampling campaigns in triplicate. Triplicate samples were used to create an overall datum for each sampling site and season. Samples were preserved in 96% ethanol for later taxonomic identification and were processed in the lab by counting and identifying all invertebrates present to the lowest possible taxonomic level using stereo-dissecting microscopes. As Chironomidae, Ceratopogonidae, and Oligochaeta were not identified below family or order level, a total of 16 taxa were distinguished in the five samplings.

For estimations of body mass, the first 100 individuals per taxon were picked from each sample and length of individuals was measured with an object micrometer to the nearest millimetre. Published taxon-specific length–mass relationships were applied to derive individual body mass as ash-free dry mass (AFDM) from length measurements (Burgherr and Meyer 1997; Benke et al. 1999; Baumgärtner and Rothhaupt 2003). In parallel to the invertebrate samplings, physical and chemical variables, i.e., water temperature, channel width, depth, discharge, current velocity and nutrient concentrations, were measured at each sampling site in each sampling campaign as described elsewhere (Gücker et al. 2006). Thus, all necessary measurements for the estimation of instantaneous secondary production (density, body mass, and temperature) were independently acquired for each sampling site in each sampling campaign.

For analyses of the stable isotope composition and elemental stoichiometry of animals and their food resources, an additional sampling campaign was conducted in July 2003 at the same sampling sites. Coarse particulate organic matter (CPOM), i.e., woody debris, leaves, and aquatic macrophytes, was collected directly from the stream and attached periphyton was removed from the CPOM prior to stable isotope analysis. The upper 1 cm of the sediments was sampled using a sediment corer (6 cm inner diameter, Uwitec, Austria) and periphyton samples were obtained from instream hard substrata, with a toothbrush. In the laboratory, periphyton samples were processed by removing detritus and invertebrates under 20× magnification using forceps. Samples of stream water nitrate and suspended POM, i.e., seston, were taken from all six sampling sites and tertiary WWTP effluents. Suspended POM contained in 300 mL streamwater was filtered on precombusted GF/C filters (nominal retention size of 1.2 µm) and dried at 60 °C for 24 h. The most dominant taxa of benthic invertebrates were sampled, identified, sorted, separated, and held alive for several hours to allow gut clearance. Molluscs were carefully removed from their shells under a stereo-dissecting microscope. Data presented for fish refers to individuals incidentally captured with the Surber sampler. Animals and POM sources were dried at 60 °C until constant weight was reached. Crustaceans were acid treated to remove inorganic carbon that could confound the carbon isotope signal of the body (Yamamuro and Kayanne 1995). Three independent samples of 1.5–3.5 mg dry mass of (*i*) each POM source (e.g., material from one leaf, twig and seston, periphyton, or sediment sample per sample), (*ii*) each invertebrate species (3–20 individuals per sample), and (*iii*) each fish species (muscle tissue from one individual per sample) were prepared for each sampling site. Samples were pulverized with

an automatic agate grinder (Pulverisette 6, Fritsch, Germany) and submitted for stable isotope and elemental analyses.

Secondary production estimates

Instantaneous secondary production (IP, mg AFDM·m^{-2}·d^{-1}) was calculated for each taxon, site, and sampling date as the product of taxon density (D, per m^2), taxon mean individual body mass (BM, mg AFDM), and instantaneous growth rate (gr, per day; Morin and Dumont 1994; Morin 1997). Instantaneous growth rate was estimated for each taxon in each sample from BM and water temperature (T, °C) at the time of sampling using an empirical model (Morin and Dumont 1994):

$$\log_{10}(gr) = -2.09 - 0.27\log_{10}(BM) + 0.025T$$

Subsequently, taxon IP values were summed up for each sample to obtain total IP of samples. The use of empirical models reduces the costs of obtaining secondary production estimates (Stephenson et al. 2007), but provides reliable estimates of annual community secondary production when applied to multispecies communities using monthly body mass and density data (Morin and Dumont 1994; Morin 1997). Considering our experimental approach that compared temporally paired estimates of community IP between agricultural and urban sites of the same catchment, our results should be robust for the purpose of such relative comparisons. Indeed, similar approaches have proven useful in studies of WWTP impacts (deBruyn et al. 2003; Singer and Battin 2007).

Chemical analyses

Organic carbon and nitrogen content of animals and POM sources were determined with an elemental analyzer (CE 1008, Thermoquest, Biberach, Germany) connected to a mass spectrometer (Delta S, Finnigan, now Thermo Electron, Waltham, Massachusetts, USA) at the stable isotope facility of the Leibniz Institute for Baltic Sea Research (Warnemünde, Germany). Details on standards, analytical procedures, and the accuracy of the measuring system have been given elsewhere (Voss et al. 2000). Carbon and nitrogen isotope data are given in the conventional delta notation as parts-per-thousand deviation from the PeeDee limestone and atmospheric N_2 standard (Peterson 1999). For total phosphorus analysis, subsamples were treated with a mixture of sulfuric acid (5 mol·L^{-1}) and hydrogen peroxide (30%) at 150 °C. Subsequently, soluble reactive phosphorus was measured by the molybdenum-blue method (Murphy and Riley 1962). Stream and wastewater nitrate-δ^{15}N was measured with an adaptation of the ammonium diffusion method (Sigman et al. 1997). Briefly, nitrate was converted into ammonia and trapped onto acidified glass-fibre filter traps. Subsequently, the filters were dried and submitted to isotopic analysis as described previously.

Data analyses

We evaluated whether differences in (*i*) density, (*ii*) individual body mass, or (*iii*) water temperature, were responsible for differences in IP estimates between agricultural and urban sites according to the empirical IP model (Morin and Dumont 1994; Morin 1997). Briefly, we evaluated the effects of each of the three variables separately from each other by fixing the values of the remaining two variables in the calculation of urban IP to the values of the respective two variables measured at agricultural sites. The estimates of IP at urban sites due to urban/agricultural differences in single variables (P'_U) obtained in this way were used to calculate an index of the effects of urban/agricultural differences in single determinants of IP (I; ranging from –1 to 1).

$$I = \frac{2 \times P'_U}{P'_U + P_A} - 1$$

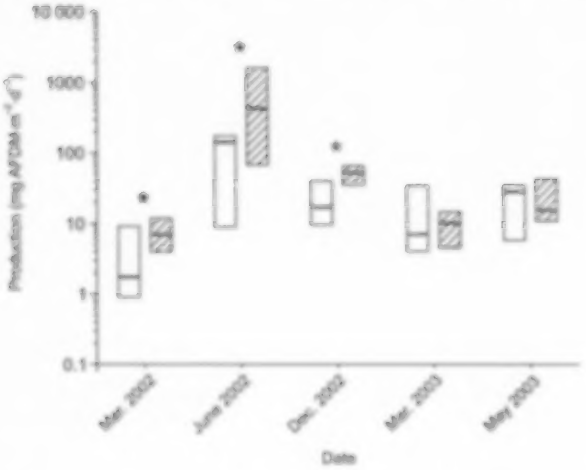
where P_A is the unmanipulated IP at agricultural sites. Index values close to –1 indicate strong negative effects of the respective determinant, i.e., much lower production at urban sites than at agricultural sites, values close to 0 indicate very weak effects and values close to +1 indicate strong positive effects on secondary production. The procedure was conducted separately for each sampling campaign using data from all recorded taxa and for each taxon exhibiting urban/agricultural differences in IP using data from all sampling campaigns. Results are separately presented for "winning" taxa, i.e., taxa with higher IP at urban sites than at agricultural sites and "losing" taxa, i.e., taxa with lower IP at urban sites than at agricultural sites.

Depending on sample distribution and variances, we conducted Wilcoxon paired-sample tests based on sampling campaign-specific median values from agricultural and urban sites, respectively, to test for differences in total IP, taxon-specific IP, and in taxon contribution to total IP between agricultural and urban sites. We used two-sample t tests (normality, equality of variances) to test for differences in stable isotope and elemental stoichiometry data between agricultural and urban sites. We explored relationships between IP of taxa, their elemental stoichiometry and stable isotope composition, as well as ratios of urban site and agricultural site IP, elemental stoichiometry, and stable isotope composition of taxa using Spearman and Pearson correlation analyses, depending on data distribution.

Contributions of particulate organic carbon (POC) and nitrate from tertiary-treated wastewater to suspended POC and stream water nitrate, respectively, of urban sites were estimated using standard linear two-source mixing models (Phillips and Gregg 2001) on δ^{15}N values of nitrate and δ^{13}C values of suspended POC. We assumed that δ^{15}N and δ^{13}C values of stream water nitrate and suspended POC, respectively, at urban sites resulted from conservative mixing of δ^{15}N and δ^{13}C of nitrate and suspended POC from upstream sources with those from tertiary-treated wastewater.

The contribution of potential food resources to the diet of macroinvertebrate consumers was determined by δ^{13}C and δ^{15}N mixing modelling with the computer program SIAR, version 4 (Stable isotope analysis in R, Jackson et al. 2009). SIAR was developed to avoid the constraints of conventional linear approaches, e.g., having more sources than tracers and lacking incorporation of uncertainties associated with consumer and resource stable isotope signatures as well as with trophic fractionation factors. The model is based on a Bayesian approach that estimates probability distributions of resource contributions to a consumer's diet by explicitly accounting for all uncertainties of the input data

Fig. 1. Medians (horizontal lines) and ranges (columns) of instantaneous invertebrate production of the three agricultural sites (open columns) and the three urban tertiary-treated wastewater-impacted sites (hatched columns). Significant differences in invertebrate production between urban and agricultural sites are indicated above the respective columns. *, $P < 0.05$, according to Mann–Whitney U tests.



(Moore and Semmens 2008). For mixing model analysis, we used all food resources potentially available to the consumers in their habitat, i.e., alder leaves, submerged macrophytes, periphyton, suspended organic matter (OM), and benthic OM. We accounted for trophic fractionation of macroinvertebrate consumers using fractionation factors published by Post (2002), i.e., 0.4 ± 1.3‰ for $\delta^{13}C$ and 3.4 ± 0.98‰ for $\delta^{15}N$. Results of the mixing model analysis are presented as mean ± 95% highest density region (hdr). The hdr is the Bayesian counterpart of confidence intervals for posterior probability distributions (Marin and Robert 2008) and is calculated by propagating all sources of uncertainty through the SIAR model (Jackson et al. 2009).

Results

Invertebrate production

Total IP (median and range over all samples taken in the five sampling campaigns) amounted to 9.6 (0.9–174) mg AFDM·m^{-2}·d^{-1} at agricultural sites and to 34.9 (4.5–1610) mg AFDM·m^{-2}·d^{-1} at urban sites. High total IP values of up to 1610 mg AFDM·m^{-2}·d^{-1} at urban sites in summer 2002 were mainly due to extremely high taxon IP values of *Dugesia tigrina* (Girard) (Turbellaria) (567 mg AFDM·m^{-2}·d^{-1}), *Bithynia tentaculata* (L.) (Gastropoda) (313 mg AFDM·m^{-2}·d^{-1}), Chironomidae (Diptera) (297 mg AFDM·m^{-2}·d^{-1}), and the invasive species *Potamopyrgus antipodarum* (Gray) (Gastropoda) (259 mg AFDM·m^{-2}·d^{-1}). Median total IP (Fig. 1) at urban tertiary-treated wastewater-impacted sites was significantly higher than that at agricultural sites (Wilcoxon paired-sample test, $P < 0.05$, $n = 5$). *Asellus aquaticus* (L.) (Crustacea), *Erpobdella octoculata* (L.) (Hirudinea) Oligochaeta, as well as *P. antipodarum* and *B. tentaculata* (Gastropoda) had significantly higher IP at urban sites than at agricultural sites (Wilcoxon paired-sample test, $P < 0.05$, $n = 5$) (Fig. 2). Chironomidae, *Pisidium* sp. (Bivalvia), and *D. tigrina* and *Radix balthica* (L.) (Gastropoda) had higher median and maximum IP at urban sites than at agricultural sites (Fig. 2); however, total IP of these taxa did not differ significantly between urban and agricultural sites (Wilcoxon paired-sample test, $P > 0.1$, $n = 5$). *Baetis vernus* Curtis (Ephemeroptera), *Gammarus pulex* (L.) and *Gammarus roeseli* (Gervais, both Crustacea) both tended to have lower IP at urban sites than at agricultural sites (Wilcoxon paired-sample test, $P < 0.1$, $n = 5$) (Fig. 2). Contributions of *A. aquaticus*, *B. tentaculata*, *E. octoculata* Oligochaeta, and *P. antipodarum*, to total IP were higher at urban than at agricultural sites (Fig. 3). In contrast, *G. pulex* and *G. roeseli*, which contributed substantially to total IP at agricultural sites (medians: 5% and 61%, respectively), had much lower median contributions to total IP at urban sites (0.1% and 9%; Fig. 3).

Water temperatures at urban sites were higher than those at agricultural sites (Table 1); thus differences in water temperature had positive effects on IP at urban sites in all sampling campaigns (Table 2). Nevertheless, differences in invertebrate density were the most important determinant of differences in IP between urban and agricultural sites both across sampling campaigns and taxa (Table 2). Interestingly, positive effects of high water temperature on the IP of *G. pulex* and *G. roeseli* at urban sites were counteracted by effects of low density and individual biomass (Table 2), resulting in lower absolute and relative values of IP of both species at urban sites (Figs. 2 and 3). In March 2003, much lower IP of *Gammarus* spp. at urban sites than at agricultural sites owing to lower mean density and individual body mass (11.5-fold and 2.2-fold, respectively) resulted in a decrease in total IP at urban sites compared to that at agricultural sites (Table 2).

Stable isotopes in particulate organic matter and dissolved inorganic nitrogen

There were profound differences in POM sources between agricultural and urban sites. In particular, allochthonous CPOM of riparian origin, such as woody debris and alder leaves, was absent at urban sites. The mean $\delta^{13}C$ value of suspended POC was significantly elevated by 3.0‰ at urban sites relative to agricultural ones (t test, $P < 0.01$, $n = 6$; Figs. 4 and 5), pointing to a shift in origin of suspended POC. The $\delta^{13}C$ values of suspended POC at urban sites and of tertiary-treated wastewater-derived POC were very similar (–27.6 ± 0.1‰ and –27.1 ± 0.1‰ respectively; mean ± SD of the three sampling sites/samples) and the latter contributed 85 ± 1% to total suspended POC at urban sites (Table 3). In contrast, mean $\delta^{13}C$ values of periphyton, sediments, and aquatic macrophytes did not differ between agricultural and urban sites (t tests, $P > 0.1$, $n = 6$).

Values of $\delta^{15}N$ of stream water nitrate, which is the dominant dissolved inorganic nitrogen (DIN) species in the investigated stream (Table 1), were elevated by 2.4‰ at urban sites (Figs. 4 and 5) compared with agricultural sites, indicating a shift in DIN sources (t test; $P < 0.01$, $n = 6$). The $\delta^{15}N$ values of urban stream water nitrate and tertiary-treated wastewater nitrate were similar (9.7 ± 0.2‰ and 9.8 ± 0.2‰, respectively; mean ± SD; t test; $P > 0.1$, $n =$

Fig. 2. Medians (horizontal lines) and ranges (columns) of differences in instantaneous invertebrate production between urban and agricultural sites for the five sampling dates. Positive values indicate higher production at urban sites. Significant differences in invertebrate production between urban and agricultural sites are indicated above the respective columns. °, $P < 0.1$ and *, $P < 0.05$, according to Wilcoxon paired-sample tests. Represented taxa contribute more than 99% to total instantaneous invertebrate production both at agricultural and urban sites.

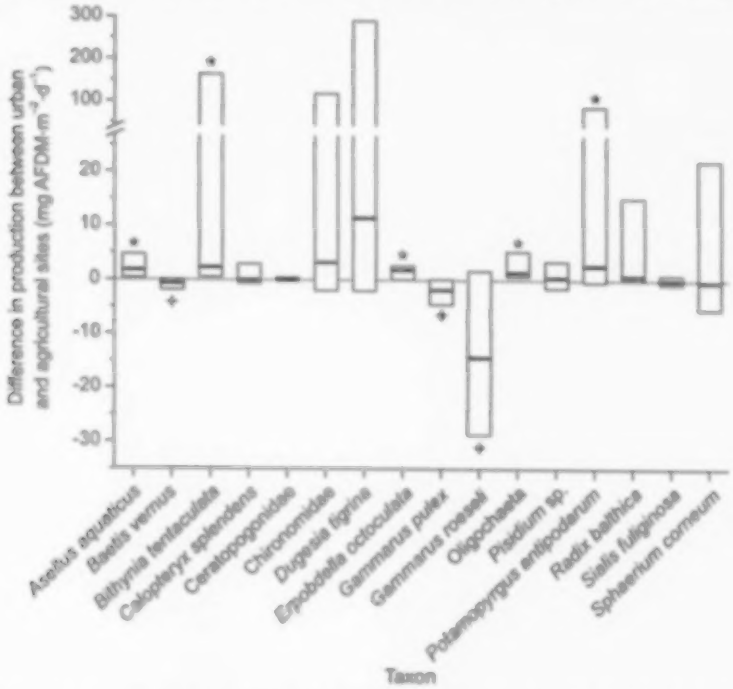


Fig. 3. Medians (horizontal lines) and ranges (columns) of the percentage contribution of taxon production to total invertebrate production per sampling date at agricultural sites (open columns) and urban tertiary-treated wastewater-impacted sites (hatched columns) for the five sampling dates. Significant differences in percentage contribution to total production between urban and agricultural sites are indicated above the respective columns. °, $P < 0.1$ and *, $P < 0.05$, according to Wilcoxon paired-sample tests. Represented taxa contribute more than 99% to total instantaneous invertebrate production both at agricultural and urban sites.

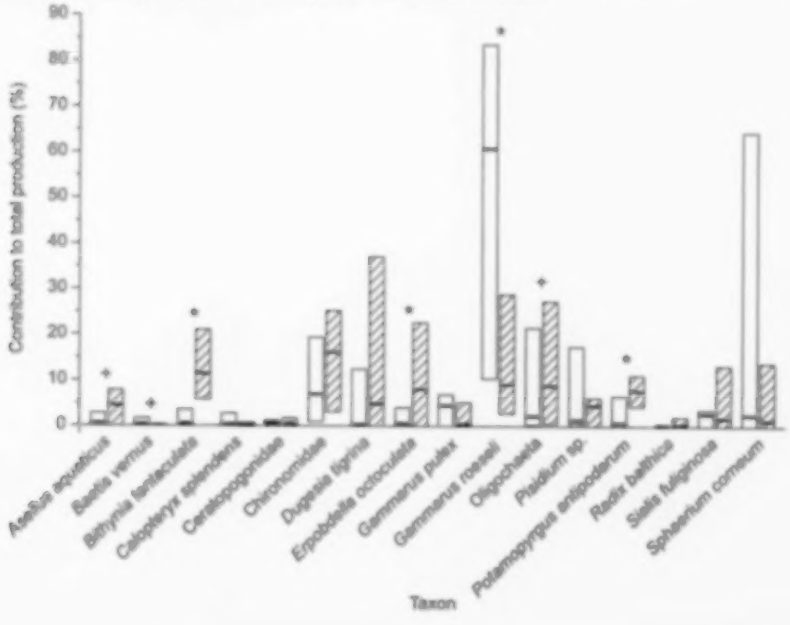


Table 2. Index of the effects of invertebrate density (*D*), individual body mass (BM), and temperature (*T*) on differences in instantaneous invertebrate production between urban and agricultural sites (*I*, as calculated by eq. 1).

	Combined effects of *D*, BM and *T*	Effect of *D*	Effect of BM	Effect of *T*
Sampling campaign				
March 2002	0.32	0.37	–0.22	0.16
June 2002	0.73	0.88	–0.47	0.06
December 2002	0.39	0.31	–0.14	0.23
March 2003	–0.54	–0.49	–0.21	0.15
May 2003	–0.01	–0.31	0.21	0.10
Winning taxa				
Asellus aquaticus	0.83	0.89	–0.20	–0.05
Bithynia tentaculata	0.99	0.98	0.51	–0.06
Chironomidae	0.78	0.84	–0.31	0.15
Dugesia tigrina	0.98	0.96	0.27	0.01
Erpobdella octoculata	0.71	0.76	–0.06	–0.05
Oligochaeta	0.44	0.22	0.15	0.09
Potamopyrgus antipodarum	0.98	0.96	0.18	0.03
Radix balthica	0.96	0.87	0.47	0.06
Losing taxa				
Baetis vernus	–0.95	–0.96	0.05	0.03
Gammarus pulex	–0.74	–0.80	–0.08	0.21
Gammarus roeseli	–0.30	–0.48	–0.01	0.21

Note: Combined effects of *D*, BM, and *T* correspond to the difference in production between urban and agricultural sites. Winning taxa are those exhibiting higher production in urban than in agricultural sites. Losing taxa are those exhibiting lower production in urban than in agricultural sites. Index values close to –1 indicate strong negative effects, i.e., much lower production at urban sites than at agricultural sites, values close to 0 indicate very weak effects and values close to +1 indicate strong positive effects. Sampling campaign-specific effects are calculated over all taxa; taxon-specific effects are calculated over all sampling campaigns.

6) and tertiary-treated wastewater nitrate contributed 96 ± 4% to total stream water nitrate at urban sites (Table 3). The $\delta^{15}N$ values of periphyton, aquatic macrophytes and sediments were also significantly elevated by 3.9‰, 5.1‰, and 3.0‰, respectively, at urban sites (*t* tests; $P < 0.01$, $P < 0.01$, $P < 0.05$, $n = 6$; Figs. 4 and 5). However, $\delta^{15}N$ values of suspended POM did not differ between urban and agricultural sites (Fig. 4) *t* test; $P > 0.1$, $n = 6$) or urban sites (Fig. 4) and tertiary-treated wastewater (9.0 ± 0.1‰) (*t* test; $P > 0.1$, $n = 6$).

Stable isotopes in secondary producers

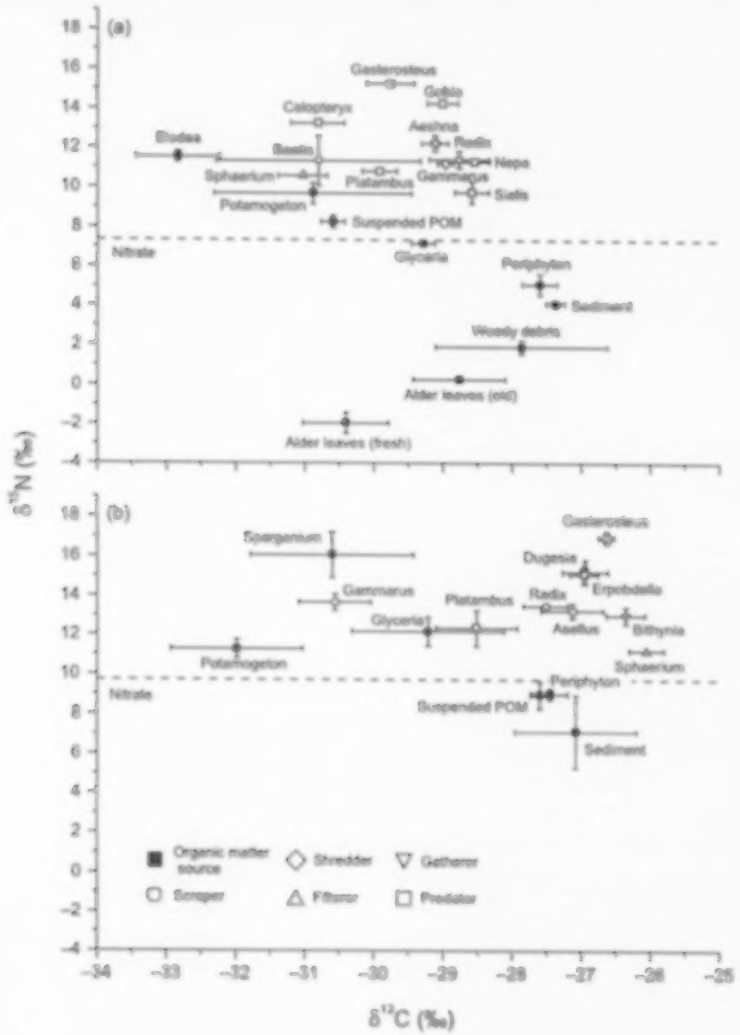
Secondary producers clearly responded to the isotopic shift in POC and DIN sources. Mean $\delta^{13}C$ and $\delta^{15}N$ values of urban secondary producers were elevated by 2.1‰ and 1.8‰, respectively, compared with secondary producers at agricultural sites (paired *t* test, $P < 0.01$, $n = 5$; Fig. 4). However, isotopic responses to urban stressors differed markedly among species. Mean $\delta^{13}C$ and $\delta^{15}N$ values of *Gasterosteus aculeatus* (L.) (Pisces) and *Platambus maculatus* (L.) (Coleoptera), both predators, were significantly elevated by 3.1‰ and 1.7‰ (*t* tests for *G. aculeatus*, $P < 0.01$, $n = 6$; Figs. 4 and 5) and 1.3‰ and 1.8‰ (*t* tests for *P. maculatus*, $P < 0.01$, $n = 6$), respectively, at urban sites compared with agricultural sites. In contrast, *S. corneum* (L.) (Bivalvia), a filterer according to its functional feeding group (FFG) classification (Schmedtje and Colling 1996), exhibited substantially higher $\delta^{13}C$ values (by 5.0‰, *t* test, $P < 0.01$, $n = 6$) at urban sites than at agricultural sites, while $\delta^{15}N$ values were only moderately increased (by 0.6‰, *t* test, $P < 0.01$, $n = 6$); thus, reflecting the change in isotopic signature of suspended POM (Figs. 4 and 5). Conversely, *G. roeseli*, a shredder with tendencies towards omnivory (Schmedtje and Colling 1996), was the only species exhibiting decreased $\delta^{13}C$ values (by –1.8‰, *t* test, $P < 0.01$, $n = 6$), but increased $\delta^{15}N$ values (by 1.6‰, *t* test, $P < 0.01$, $n = 6$) at urban sites compared to agricultural sites.

Resource use of secondary producers and mixing model analysis

According to mixing model analyses, the primary consumers encountered in the stable isotope sampling campaign were surprisingly omnivorous (Table 4). For example, *S. corneum*, an exclusive filter feeder according to its FFG classification (Schmedtje and Colling 1996), appeared to assimilate considerable amounts of sediment and periphyton (Table 4). *Asellus aquaticus* and *G. roeseli*, both shredders with tendencies towards omnivory according to their FFG classification (Schmedtje and Colling 1996), did not appear to exhibit clear preferences for CPOM (Table 4).

Mixing model analysis suggested that instream alder CPOM, which had only been found at agricultural sites, contributed only to a minor degree to the primary consumers' diet (Fig. 6*a*), but was the most important primary resource contributing to secondary consumers' diet at agricultural sites (Fig. 6*b*). Conversely, sediment OM was the most im-

Fig. 4. Stable isotope biplots of food sources (filled squares) and consumers (open symbols) at (*a*) agricultural and (*b*) urban tertiary-treated wastewater-impacted sites. $\delta^{15}N$ and $\delta^{13}C$ values are given as means ±1 SD of all samples. $\delta^{15}N$ of stream water nitrate is represented as a broken line.



portant diet of secondary consumers at urban sites (Fig. 6*b*). In general, CPOM tended to contribute less, whereas periphyton and sediment organic matter tended to contribute more to the macroinvertebrate community's diet at urban sites than at agricultural sites.

Elemental stoichiometry of particulate organic matter sources and secondary producers

The mean molar C:N:P ratio of all POM sources was 1008:70:1 at agricultural sites and 235:25:1 at urban sites. Molar C:N, C:P, and N:P ratios of POM were significantly higher at agricultural sites than at urban sites (*t* tests; $P < 0.05$, $P < 0.01$ and $P < 0.05$, $n = 15$, respectively; Fig. 7). Hence, mean molar P and N contents of POM at urban sites were 4.3 and 1.7 times, respectively, higher at urban sites than at agricultural sites. Sediment and periphyton exhibited highest degrees of P enrichment with molar P contents 19× and 8× higher at urban sites than at agricultural sites (Fig. 7). Higher mean molar N contents of POM of urban sites were mainly due to the absence of allochthonous POM sources with low N contents (i.e., woody debris and alder leaves) at these sites (Fig. 7). Suspended POM at urban sites was only moderately enriched in N and P with mean molar N and P contents 1.3× and 2.1× higher than those at agricultural sites.

The mean molar C:N:P ratio of secondary producers was 140:27:1 at agricultural sites and 155:30:1 at urban sites. In contrast to molar C:N, C:P, and N:P ratios of food resources, those of secondary producers did not differ significantly between urban and agricultural sites (*t* tests, $P > 0.1$, $n = 20$, Fig. 7). No species exhibited significant differences in its elemental stoichiometry between urban and agricultural sites (*t* tests, $P > 0.1$, $n = 6$).

Fig. 5. Stable isotope biplot depicting significant changes (*t* tests, $P < 0.05$, $n = 6$) in food source (broken arrows) and consumer (unbroken arrows) isotopic composition between agricultural and urban tertiary-treated wastewater-impacted sites. The position of the origin of each arrow is the mean isotopic composition at agricultural sites; the position of the head of each arrow is the mean isotopic composition at urban sites.

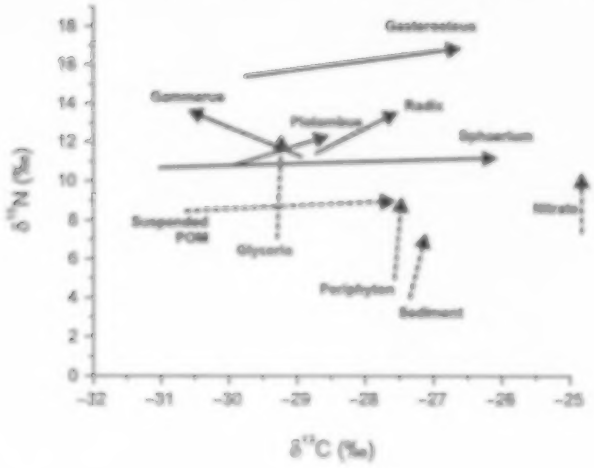


Table 3. Structure and results of end member mixing analyses (EMMA).

EMMA	Sources	Source contribution to mixture (%)
Urban seston POC		
Two source, single isotope (^{13}C)	TTWW POC	85.2±1.4
	Agricultural seston POC	14.8±1.4
Urban streamwater NO_3^-		
Two source, single isotope (^{15}N)	TTWW NO_3^-	96.4±3.5
	Agricultural streamwater NO_3^-	3.6±3.5

Note: Source contributions are given as means ± 1 SD. Stable isotope signatures of mixtures and sources are given in Fig. 4. Models and calculation of SDs according to Phillips and Gregg (2001). TTWW, tertiary-treated wastewater.

Relationships between secondary production, stable isotope composition and elemental stoichiometry of secondary producers

There were no significant (Spearman or Pearson correlations, $P < 0.05$) relationships between IP of taxa, their elemental stoichiometry and stable isotope composition as well as ratios of urban site and agricultural site IP, elemental stoichiometry and stable isotope composition of taxa.

Discussion

Secondary production

Historically, invertebrate secondary production of urban streams was reduced by massive inputs of crudely treated organically rich wastewater (Hynes 1974; Welch and Lindell 1992; Zelinka 1977). However, up to 210-fold increases in chironomid production, accompanied by changes in community composition and decreases in chironomid diversity, were reported as a result of organic pollution in Czech mountain streams (Losos 1982). In general, moderate increases in secondary production were found where inputs of primary- and secondary-treated wastewater were small relative to stream discharge and did not lead to extensive hypoxia (deBruyn et al. 2003; Shieh et al. 2003; Singer and Battin 2007). In our study, total instantaneous secondary production of an agricultural lowland stream increased due to substantial discharge of tertiary-treated wastewater and secondary effects such as stimulated instream primary productivity. As other urban stressors, such as channel straightening and incision, altered hydrology, or pesticide use can be assumed to have adverse effects on secondary productivity (Paul and Meyer 2001, Walsh et al. 2005), tertiary-treated wastewater discharge appears to be the dominant urban stressor in the investigated stream.

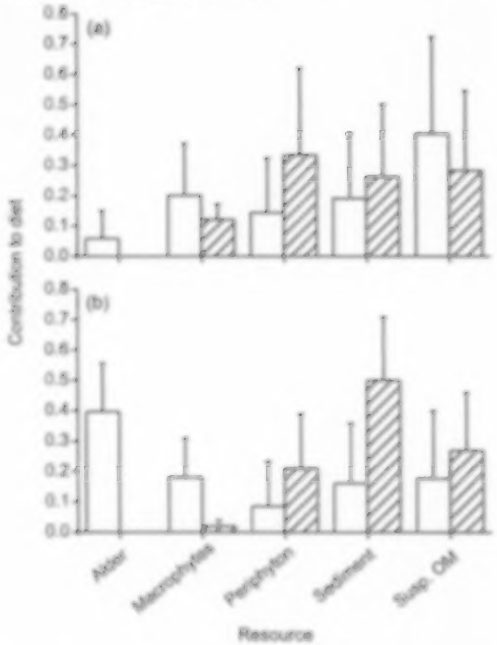
The 1.4- to 6.8-fold increases in median instantaneous secondary production in our study (note in Fig. 1 the missing increases in 2003 due to production-decreases of shredders) is in the same order of magnitude as the production increases reported for the fifth-order river, La Poudre, in Colorado, which receives a moderate discharge of secondary-treated wastewater (2.4-fold production increase) (Shieh et al. 2003), and for a third-order forest stream in Austria, which receives small inputs of secondary-treated wastewater (1.2- to 2.6-fold production increase) (Singer and Battin 2007), as well as for urban sites in the effluent dispersion plume of the City of Montreal's primary WWTP (1.8- to 4.1-fold production increase) (deBruyn et al. 2003). Considering differences in the contribution of wastewater discharge to stream

Table 4. Mean (and 95% highest density region) contribution of food resources to the body mass of macroinvertebrate primary and secondary consumers at the agricultural (agri) and urban tertiary-treated wastewater-impacted (urban) sites.

	Alder		Macrophytes		Periphyton		Sediment		Suspended OM	
	Agri	Urban	Agri	Urban	Agri	Urban	Agri	Urban	Agri	Urban
Primary consumer										
Asellus aquaticus	—	—	—	0.10 (0.04–0.17)	—	0.35 (0.09–0.60)	—	0.24 (0.00–0.46)	—	0.30 (0.05–0.54)
Baetis vernus	0.09 (0.00–0.24)	—	0.31 (0.10–0.52)	—	0.16 (0.00–0.35)	—	0.19 (0.00–0.39)	—	0.25 (0.00–0.47)	—
Bithynia tentaculata	—	—	—	0.12 (0.01–0.22)	—	0.33 (0.03–0.59)	—	0.25 (0.00–0.47)	—	0.30 (0.01–0.54)
Gammarus roeseli	0.18 (0.00–0.37)	—	0.31 (0.11–0.52)	0.32 (0.12–0.55)	0.27 (0.00–0.51)	0.35 (0.03–0.64)	0.24 (0.00–0.48)	0.33 (0.00–0.61)	—	—
Radix balthica	0.13 (0.00–0.29)	—	0.22 (0.03–0.39)	0.18 (0.03–0.34)	0.21 (0.00–0.4)	0.29 (0.01–0.53)	0.20 (0.00–0.40)	0.25 (0.00–0.47)	0.24 (0.00–0.44)	0.28 (0.01–0.52)
Sphaerium corneum	—	—	—	—	0.22 (0.00–0.46)	0.27 (0.00–0.55)	0.29 (0.00–0.56)	0.41 (0.04–0.76)	0.49 (0.23–0.79)	0.31 (0.00–0.61)
Secondary consumer										
Aeshna sp.	0.25 (0.12–0.39)	—	0.12 (0.01–0.25)	—	0.19 (0.00–0.36)	—	0.19 (0.00–0.38)	—	0.25 (0.02–0.45)	—
Calopteryx splendens	0.18 (0.03–0.33)	—	0.18 (0.01–0.34)	—	0.18 (0.00–0.37)	—	0.19 (0.00–0.40)	—	0.26 (0.02–0.49)	—
Dugesia tigrina	—	—	—	0.02 (0.00–0.04)	—	0.23 (0.02–0.44)	—	0.45 (0.18–0.72)	—	0.30 (0.07–0.53)
Erpobdella octoculata	—	—	—	0.03 (0.00–0.06)	—	0.26 (0.01–0.49)	—	0.40 (0.09–0.71)	—	0.32 (0.04–0.56)
Nepa cinerea	0.29 (0.16–0.43)	—	0.06 (0.00–0.13)	—	0.22 (0.01–0.4)	—	0.21 (0.00–0.41)	—	0.22 (0.03–0.39)	—
Sialis fuliginosa	0.41 (0.23–0.61)	—	0.04 (0.00–0.11)	—	0.19 (0.00–0.41)	—	0.24 (0.00–0.47)	—	0.12 (0.00–0.27)	—

Note: Contributions were calculated using a five-source mixing model in the SIAR software (Jackson et al. 2009).

Fig. 6. Mean (±95% highest density region) contribution of food resources to the body mass of macroinvertebrate (*a*) primary consumers and (*b*) secondary consumers at agricultural (open columns) and urban tertiary-treated wastewater-impacted sites (hatched columns). Contributions were calculated using a five-source mixing model in the SIAR software (Jackson et al. 2009). Susp. OM, suspended organic matter. See Table 4 for contributions of food resources to the diet of each consumer.



discharge between our study and the aforementioned studies, tertiary-treated wastewater discharge in our study appears to affect stream secondary production less severely than inputs of primary- and secondary-treated wastewater reported in the literature. Production increases due to massive tertiary-treated wastewater discharge in our study were comparable with those due to small and moderate inputs of primary- and secondary-treated wastewater in the literature, suggesting that adequate dilution of tertiary-treated wastewater can further mitigate adverse effects on stream ecosystem functioning.

The upper range of total instantaneous secondary production at the urban sites studied in summer 2002 (1610 mg AFDM·m^{-2}·d^{-1}) was high compared with published annual secondary production rates when converted to diel rates. However, the median total instantaneous secondary production calculated over five seasonal sampling campaigns for both agricultural (9.6 mg AFDM·m^{-2}·d^{-1}) and urban sites (34.9 mg AFDM·m^{-2}·d^{-1}) was in the lower part of the range of annual secondary production rates typically reported in the literature (approx. 3–2500 mg AFDM·m^{-2}·d^{-1}; Benke 1993; Huryn and Wallace 2000). Although the comparison of our instantaneous secondary production rates with published annual secondary production rates is inherently problematic, this comparison may indicate that the investigated urban and agricultural study sites exhibit low secondary production values, especially when considering their high nutrient concentrations and primary production rates (Gücker et al. 2006).

Various taxa of different FFGs, especially predators, exhibited higher contributions to total community production at urban sites than at agricultural sites. In particular, pollution-tolerant taxa (Schmidt-Kloiber et al. 2006), such as *D. tigrina*, *A. aquaticus*, *P. antipodarum*, *B. tentaculata*, and *E. octoculata*, were favoured by urban stressors. However, *G. roeseli* and *G. pulex*, both shredders with a tendency towards omnivory according to their FFG classification (Schmedtje and Colling 1996), exhibited lower production and lower contributions to total invertebrate production at urban sites than at agricultural sites. Interestingly, differences in instantaneous invertebrate production between urban and agricultural sites were mainly due to changes in invertebrate density and body mass, and only to a minor degree due to differences in water temperature, highlighting the potential role of food availability, quantity, and quality, as determinants of secondary production in urban streams. These results from urban sites impacted by substantial discharge of tertiary-treated wastewater partially correspond with other studies of urban stream reaches receiving moderate inputs of primary- and secondary-treated wastewater, in which increased production of gatherers (Shieh et al. 2003; Singer and Battin 2007), filterers (deBruyn et al. 2003; Shieh et al. 2003) and predators (Singer and Battin 2007), but decreased production of shredders was found (deBruyn et al. 2003; Shieh et al. 2003).

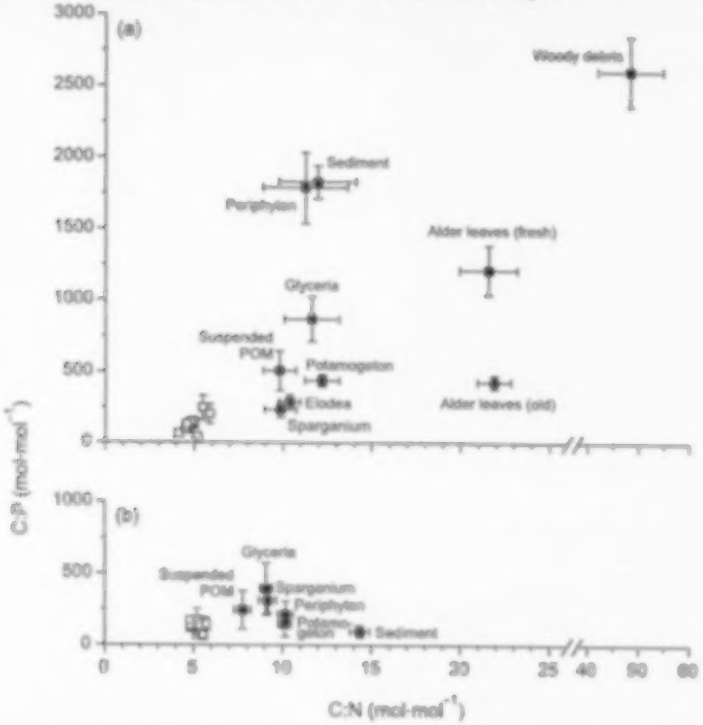
Sources of particulate organic matter and dissolved inorganic nitrogen

The contemporary urban stressors that were investigated, left obvious isotopic and stoichiometric signals in the POM pool of the studied agricultural lowland stream. POM sources at urban sites had higher molar P and N contents than POM sources at agricultural sites. These differences were likely affected by contributions of P- and N-enriched wastewater-derived POM, as well as higher SRP and DIN concentrations that affected primary production and (or) the absence of low-N allochthonous CPOM stocks at urban sites (qualitative results).

The increase in ecosystem $\delta^{13}C$, i.e., the mean $\delta^{13}C$ of all POM including invertebrates, at urban sites was likely the result of the large contribution (85% according to end member mixing analyses, and 71% according to annual flux calculations) of high-$\delta^{13}C$, tertiary-treated wastewater-derived POM to suspended POM at urban sites. Urban sites exhibited $\delta^{13}C$ values of suspended POM, which were 3‰ higher than those at agricultural sites. Similarly, increases in $\delta^{13}C$ of suspended POM of about 1–2‰, owing to the discharge of primary- and secondary-treated wastewater, have been reported (deBruyn and Rasmussen 2002; deBruyn et al. 2003; Ulseth and Hershey 2005). However, Northington and Hershey (2006) did not find significant differences in $\delta^{13}C$ of suspended POM due to the discharge of secondary-treated wastewater. Differences in the contributions of wastewater discharge to stream discharge and in organic carbon removal efficiencies of WWTPs appear to drive differences in $\delta^{13}C$ of suspended POC between the aforementioned studies.

Interestingly, wastewater-derived POM did not exhibit higher (Ulseth and Hershey 2005; Singer and Battin 2007)

Fig. 7. Nutrient stoichiometry of food sources (filled squares) and consumers (open squares) at (*a*) agricultural and (*b*) urban tertiary-treated wastewater-impacted sites. Molar C:N and C:P ratios are given as means ±1 SD of all samples.



or lower $\delta^{15}N$ values than upstream ambient suspended POM (deBruyn and Rasmussen 2002; deBruyn et al. 2003). Thus, elevated ecosystem $\delta^{15}N$ at the urban sites was likely a result of the assimilation of tertiary-treated wastewater-derived DIN with relatively high $\delta^{15}N$ values in combination with the absence of riparian inputs of low-$\delta^{15}N$ CPOM (qualitative results). Wastewater-derived DIN can generally be expected to exhibit high $\delta^{15}N$ values because of the high trophic position of humans and losses of ^{14}N due to ammonia volatilization and denitrification (Heaton 1986; DeSimone and Howes 1996; Deutsch et al. 2005).

Elemental stoichiometry and secondary production

Nutrient ratios of POM sources and secondary producers, as well as consumer-resource nutrient imbalances found in our study, corresponded well with values previously reported for benthic stream and lake communities (Cross et al. 2003; Frost et al. 2003; Liess and Hillebrand 2005). According to molar C:P and N:P ratios, P content of organic matter was higher at urban sites than at agricultural sites. However, secondary producers were homeostatic, and did not respond by incorporating additional P into their biomass at urban sites. Increases in secondary production in our study could thus result from reduced metabolic costs associated with maintaining elemental homeostasis at urban sites, such as decreased ingestion and digestion activity. This hypothesis is supported by the fact that sediment OM and periphyton exhibited the highest increases in P content, owing to urban stressors, and that taxa primarily assimilating OM from these two sources, (in particular, *A. aquaticus*, *B. tentaculata*, *D. tigrina* and *E. octoculata*) contributed to the higher community secondary production at urban sites than at agricultural sites. Alternatively, increased primary production (Gücker et al. 2006), organic C availability, as well as increased organic C quality at urban sites could be the reason for increases in secondary production. It is notable that production increases due to the studied urban stressors were, to a minor degree, due to increased streamwater temperatures at urban sites. The relative importance of effects of trophic resource and water temperature changes due to urbanization on secondary production, and especially the potential interactions of both effects, cannot be exactly assessed based on observational data like that presented in our study. However, this may be an interesting research question for future experimental work on secondary production in urban streams.

Resource use of secondary producers

Secondary producers clearly responded to the observed shift in food resources with substantial increases in whole-community $\delta^{13}C$ and $\delta^{15}N$ values. Tertiary-treated wastewater-derived POC entered the stream's food web through various filter-feeding and omnivorous taxa. For example, *S. corneum* exhibited high increases in $\delta^{13}C$ values, corresponding to the large contribution of primarily tertiary-treated wastewater-derived urban seston to its diet.

It is also notable that the community's increase in $\delta^{13}C$, owing to the studied urban stressors, may have been damp-

ened by a decrease in $\delta^{13}C$ values of omnivorous shredders, such as *G. roeseli* and *A. aquaticus*, whose allochthonous high-$\delta^{13}C$ food sources were absent at urban sites, probably as a result of riparian clearcutting, and perhaps also as a result of the often-reported flashier hydrology at urban sites (Walsh et al. 2005). *Gammarus roeseli*, the most important primary consumer at our agricultural study sites, was able to adapt its diet in response to urbanization-related changes in CPOM availability. However, this change in *G. roeseli*'s diet and — perhaps more importantly, considering the surprisingly small contribution of CPOM to *G. roeseli*'s diet — the loss of CPOM as a habitat, were associated with a dramatic reduction of its secondary production, which corresponds well with results from population growth experiments (Pöckl 1995). Consequently, urban stressors may have impacts on ecosystem functions provided by species even though species richness remains unaltered.

Interestingly, allochthonous alder CPOM contributed to the body mass of primary consumers only to a minor degree, but was the most important carbon source to secondary consumers' biomass at agricultural sites. Thus, macroinvertebrate predators either preferentially assimilated alder-derived carbon from their prey's biomass, or more likely, we missed important alder-derived carbon-consuming prey taxa in our stable isotope (SI) sampling campaign. Indeed, in our SI sampling campaign, we only encountered several important primary consumer taxa, such as Chironomidae and Oligochaeta, in biomasses too low to be submitted to SI analysis. In the nearby fifth-order river Spree, alder CPOM contributed 27% (4%–48%; mean and 95% hdr) to the biomass of Chironomidae and 26% (6%–44%) to the biomass of Oligochaeta, according to stable isotope analyses and three-source mixing models (D. Graeber, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany, personal communication 2009). As Chironomidae and Oligochaeta contributed 6% (1%–19%; median and ranges) and 2% (0.2%–22%), respectively, to total instantaneous secondary production at agricultural sites in our study, preferential predation on Chironomidae and Oligochaeta by secondary consumers may explain the high contribution of alder to the biomass of secondary consumers at agricultural sites. Accordingly, it is possible that our analysis of secondary producers' food resource use greatly underestimates the role of allochthonous food resources at agricultural sites.

The strong elevation of the urban community's $\delta^{15}N$ value in our study appeared to be a combined effect of eutrophication, i.e., inputs of tertiary-treated wastewater-derived DIN with a high $\delta^{15}N$ value and the absence of riparian low-$\delta^{15}N$ CPOM owing to riparian clearcutting and possibly also a flashier hydrology at urban sites (qualitative results). Urban periphyton and macrophyte $\delta^{15}N$ values were substantially elevated, probably owing to the incorporation of tertiary-treated wastewater-derived DIN into their biomass. Biomass of primary producers in turn was efficiently assimilated by the stream's food web. While other studies have emphasized the entry of secondary-treated wastewater-derived PON into stream food webs (Northington and Hershey 2006; Singer and Battin 2007), our study points to the importance of tertiary-treated wastewater-derived DIN for stream primary and secondary production. POM produced by and subsequently exported from urban streams may thus contribute to the elevated $\delta^{15}N$ values of POM observed in downstream estuarine and marine systems (Voss et al. 2000).

In this study, present-day urban stressors, such as substantial tertiary-treated wastewater discharge and riparian clearcutting, affected secondary producers less severely than historically recorded, but still changed matter fluxes and ecosystem functioning of an agricultural lowland stream. The trophic basis of stream secondary producers shifted from natural and agricultural resources to urban resources, i.e., POM from tertiary wastewater treatment as well as autochthonous primary production based on DIN from tertiary wastewater treatment. Resulting changes in functional properties of the invertebrate community, owing to urban stressors, such as production increases of pollution-tolerant taxa or production decreases of taxa partially depending on allochthonous CPOM habitats, may have important implications for higher trophic levels not only within the stream, but also in the riparian zone (Nakano and Murakami 2001; Paetzold et al. 2005). Changes in taxon-specific secondary production may also affect other invertebrate-mediated ecosystem functions, such as nutrient mineralization (Hall et al. 2007).

Here, we reported effects of common urban stressors on an already impacted eutrophic agricultural stream, which is the type of stream most typically found in the surroundings of large cities. Thus, even stronger effects of contemporary urbanization could potentially be found when comparing urban streams with less impaired ones. As the resource use and secondary production of the most important secondary producer in our study, *G. roeseli*, changed in response to the absence of allochthonous CPOM habitats, probably owing to riparian clearcutting and altered hydrology, our results may support previous findings that the restoration of the native riparian vegetation and a more natural stream morphology and hydrology may have important beneficial effects on urban streams (Northington and Hershey 2006; Bukaveckas 2007). Adequate dilution of tertiary-treated wastewater may mitigate effects of massive tertiary-treated wastewater discharge on total community secondary production and especially on the production of pollution-tolerant taxa.

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A steep genetic cline in yellowtail rockfish, *Sebastes flavidus*, suggests regional isolation across the Cape Mendocino faunal break

Jon E. Hess, Russell D. Vetter, and Paul Moran

Abstract: As with all *Sebastes* species, yellowtail rockfish (*S. flavidus*) produce larvae with an extended pelagic juvenile phase that can be advected in coastal currents. While dispersal potential is high, previous research on population genetic characteristics of *Sebastes* species indicates that apparent realized dispersal can be much lower and can exhibit complex patterns of genetic structure. We assayed 812 bp of the mitochondrial cytochrome *b* gene and six microsatellite loci in ~1000 yellowtail rockfish collected from 21 sites that span the species range from southern California to southeastern Alaska. An abrupt genetic cline near Cape Mendocino, California, splits the range into a northern and southern stock, and is highly concordant between our mitochondrial (F_{CT} = 0.32, $p \ll 0.001$) and microsatellite (F_{CT} = 0.02, $p \ll 0.001$) datasets. We show that this pattern may be due to a combination of physical (oceanographic or other barriers to larval dispersal), biological (habitat differences), and historical events. This study, and both intra- and inter-specific evidence from other marine species suggests Cape Mendocino demarcates two regions experiencing divergent evolutionary trajectories, and should be considered in management strategies.

Résumé : Comme c'est le cas pour toutes les espèces de *Sebastes*, le sébaste à queue jaune (*S. flavidus*) produit des larves à phase juvénile pélagique prolongée qui peuvent être entraînées par advection dans les courants côtiers. Bien que le potentiel de dispersion soit élevé, des études antérieures sur les caractéristiques génétiques des espèces de *Sebastes* indiquent que la dispersion réalisée apparente peut être beaucoup plus faible et qu'il peut y avoir des patrons complexes de structure génétique. Nous avons analysé 812 pb du gène mitochondrial du cytochrome *b* et six locus microsatellites chez ~1000 sébastes à queue jaune récoltés dans 21 sites qui couvrent l'ensemble de l'aire de répartition de l'espèce du sud de la Californie au sud-est de l'Alaska. Un cline génétique abrupt près du cap Mendocino, Californie, divise l'aire de répartition en stocks du nord et du sud et ce cline est appuyé de façon hautement concordante par nos banques de données mitochondriales (F_{CT} = 0,32; $p \ll 0,001$) et microsatellites (F_{CT} = 0,02; $p \ll 0,001$). Nous démontrons que ce patron peut être dû à une combinaison de causes physiques (barrières océaniques ou autres à la dispersion des larves), biologiques (différences d'habitat) et historiques. Notre étude et des données intra- et interspécifiques provenant d'autres espèces marines indiquent que le cap Mendocino représente la démarcation entre deux régions qui connaissent des trajectoires évolutives divergentes, ce dont on devrait tenir compte dans les stratégies de gestion.

[Traduit par la Rédaction]

Introduction

While it was originally anticipated that marine species with protracted pelagic larval stages would be genetically homogenized across much of their ranges (Palumbi 1994), subsequent research on many marine species has indicated that the marine metapopulation concept (Kritzer and Sale 2006) may be a more appropriate model for understanding their evolutionary biology and ecology. Even in species with extensive pelagic juvenile phases, behaviorally mediated dispersal (Gerlach et al. 2007) and entrainment in contemporary oceanographic features (Gilg and Hilbish 2003) can lead to high levels of self-recruitment and significant barriers to gene flow. In addition to contemporary patterns of dispersal, a broad range of factors can contribute to genetic structure in marine species, including natural selection (Gaggiotti et al. 2009), extinction-recolonization (Grant and Bowen 1998), sea-level change (Earl et al. 2010), and harvest policies (Hu and Wroblewski 2009). It is important to identify the relative importance of the various evolutionary processes underpinning the genetic patterns observed within a particular species to understand the relevance of a species' genetic structure to the marine community to which it belongs. For instance, knowing whether genetic divergence ob-

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J.E. Hess.[1] Columbia River Inter-Tribal Fish Commission, 3059-D National Fish Hatchery Road, Hagerman, ID 83332, USA.
R.D. Vetter. Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, CA 92037-1508, USA.
P. Moran. Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, WA 98112-2097, USA.

[1]Corresponding author (e-mail: hesj@critfc.org).

Fig. 1. Map of yellowtail rockfish collections, mtDNA 1-step clade frequencies, nested haplotype network, and sample sizes. The inset map in grey scale shows North America with a box around the region of interest. The colors in the pie-charts correspond to the haplotypes at top of figure. Collection sample sizes are shown above each chart and numbers below charts refer to collection names. The depth range highlighted in yellow, is the maximum depth where yellowtail are most abundant and red marks the deepest depth range yellowtail have been recorded. The nested clade showed significant patterns at the two-step level within clade 2-2 (range expansion) and at the total cladogram level (range expansion).

served between two populations resulted from a sudden dramatic change in population size versus an ongoing dispersal barrier, is essential to predict how species other than the one being observed may be influenced. In this scenario, the ongoing dispersal barrier might be predicted to affect other sympatric species and exert an influence into the future, whereas historic changes in population size may be less likely to be widely applicable across species, depending on the degree of similarity of life histories and systematic relationships among species under consideration. Population genetic structure that relates to contemporary patterns of recruitment and population connectivity is central to a biological foundation for regional fishery management, including the design of conservation measures such as marine protected area (MPA) networks.

The rockfishes (genus *Sebastes*) are the most species-rich and ecologically diverse group of marine fishes inhabiting the temperate rocky reefs of the west coast of North America (Love et al. 2002; Gunderson and Vetter 2006). As such, they are particularly appealing to study to advance our understanding of how historical and contemporary evolutionary processes interact with life-history traits, systematic relationships, and the environment to produce patterns in genetic structure. Most species occupy discrete rocky habitats separated by soft sediments, thus fitting the definition of marine metapopulations (Hanski and Gilpin 1997; Gunderson and Vetter 2006; Kritzer and Sale 2006). The adults of most rockfish species are nonmigratory and depend on the pelagic dispersal of juveniles as a primary means of colonization and population connectivity (Love et al. 2002; Starr et al. 2002; Parker et al. 2007).

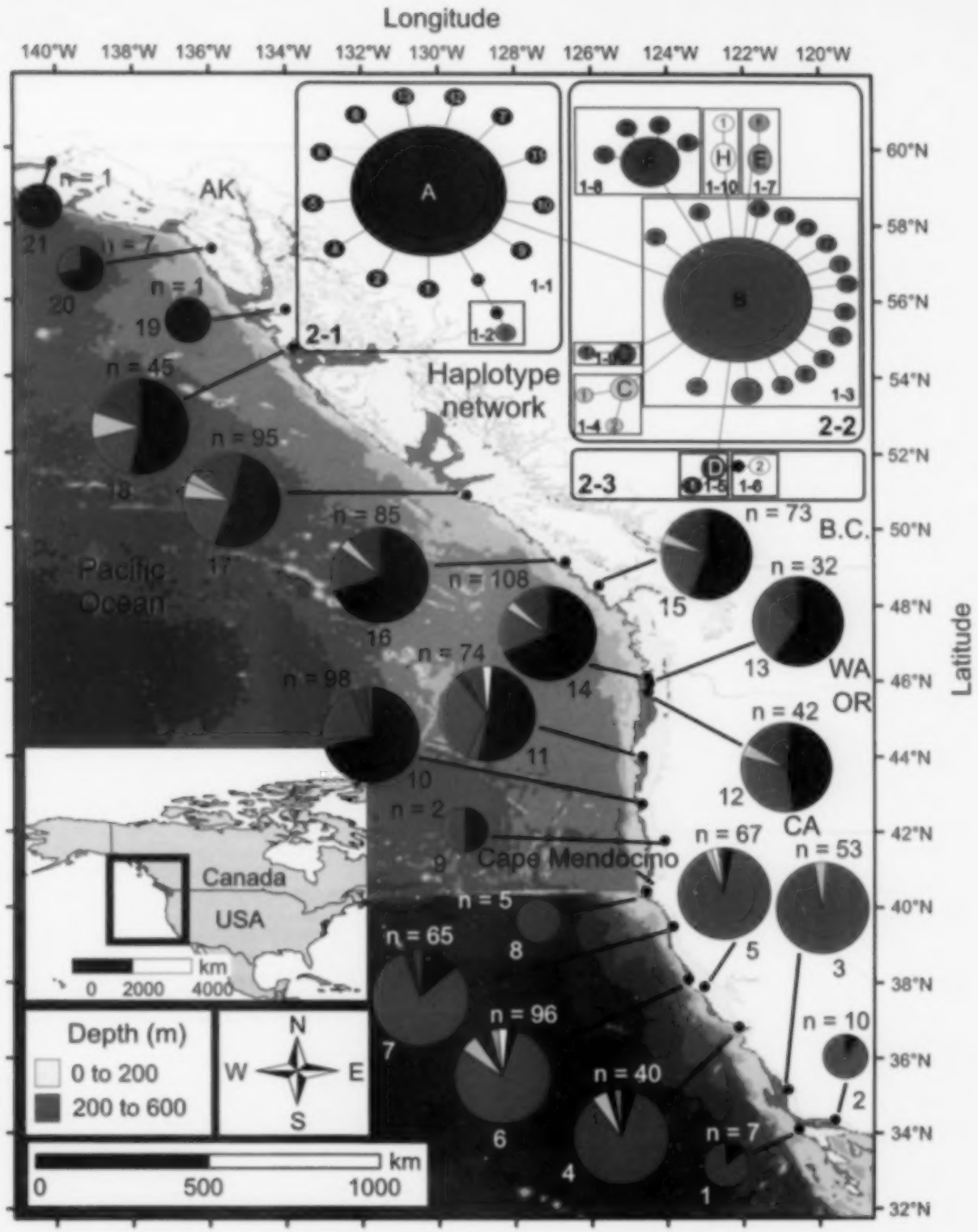
Population geneticists face several challenges in studying and interpreting the genetic structure of marine species in a way that is relevant to management issues. The detection of genetic structure is made difficult, because marine populations typically have high effective population size (*Ne*) and so genetic divergence (e.g., F_{ST}) will remain small, even when populations are completely isolated and share no migrants for many generations (Nei and Chakravarti 1977). In addition, what little genetic divergence is observed can be generated by both historical and contemporary processes, although the effects of the former can confound the measure that interests managers the most, contemporary dispersal. We can distinguish historical from contemporary processes that likely influenced genetic structure by combining two approaches: an analysis using gene-flow models based on *F* statistics (Wright 1943), and nested clade phylogeographical analysis (NCPA) using mitochondrial sequences (Templeton 2009*a*).

Our goal was to examine the population genetics of one rockfish species and test the null hypothesis of panmixia. Our secondary goal was to determine whether departure from panmixia could be entirely attributed to one of two models of contemporary processes; specifically, isolation by distance versus barrier to gene flow. In addition, we examined evidence for the influence of historical events on genetic structure. We chose to study yellowtail rockfish, *Sebastes flavidus*, which belongs to the subgenus *Sebastosomus*. Redefined by Hyde and Vetter (2007), *Sebastosomus* contains five species (blue, widow, olive, black, and yellowtail rockfishes) that tend to form schools or aggregations associated with high-relief habitats. This assemblage is also the "structure schooling ecological group" (Lenarz et al. 1995). Yellowtail rockfish are most common as adults over rocky reefs at depths from 90 to 180 m (Love et al. 2002). Although they appear to be highly sedentary as adults (Stanley et al. 1994; Lea et al. 1999; Parker et al. 2007), there has been at least one tag return showing movement over 1000 km (Love et al. 2002). Its latitudinal range includes northern Baja California, Mexico, to southeastern Alaska. This species has peak parturition of larvae in winter (Moser 1996), maintains a pelagic juvenile phase of 3–4 months (Love et al. 2002), and settles in the kelp canopy (Ammann 2004).

Yellowtail rockfishes are managed primarily by the Pacific Fisheries Management Council (PFMC) as a high value commercial fishery. They are also important in deeper water recreational fisheries. The PFMC recognizes two stocks separated by Cape Mendocino at latitude 40°30′N. Most of the US commercial catch occurs north of Cape Mendocino, California, in the northern stock distribution that extends to southern Vancouver Island, British Columbia.

There have been two published genetic studies conducted on portions of the range of yellowtail rockfish (Wishard et al. 1980; McGauley and Mulligan 1995); however, so far, no comprehensive range-wide surveys have been completed. An allozyme study (Wishard et al. 1980) and a restriction length polymorphism (RFLP) analysis of the mitochondrial 16S ribosomal gene (McGauley and Mulligan 1995) did not detect significant population genetic differences among samples from Northern California to Vancouver Island. Neither of these studies found regional breaks that would suggest more than one genetic stock is present in this species.

In this study we examine patterns of genetic diversity and population genetic differences in over 1000 *S. flavidus* individuals from 21 collection sites spanning the entire range where animals are landed in significant numbers. Mitochondrial DNA (mtDNA) sequence information was obtained from an 812 base pair (bp) portion of the cytochrome *b* gene, and nuclear DNA was examined by genotyping six microsatellite (μsat) loci. Our analyses were designed not only to test for significant stock structure, but also to discriminate the potential contemporary and historical processes involved. Identification of the evolutionary processes affecting this rockfish species will contribute to our general understanding of the forces that have shaped the *Sebastes* species flock.


Longitude
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132°W
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60°N
58°N
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Materials and methods

Sampling

We obtained muscle and fin tissue from 1013 individual fish, which represent 21 collection sites (Fig. 1 and Table 1), from multiple sources between 1993 and 2005. The majority of the samples were from the US National Oceanic and Atmospheric Administration (NOAA) groundfish trawl surveys. However, collection 17 originated from a Canadian Department of Fish and Oceans (DFO) trawl survey, and two collections (3 and 7) were collected from recreational vessels in 2005.

Molecular methods

Tissues were stored in 95% ethanol before DNA was extracted using DNeasy 96 Tissue Kits with a BioRobot 8000 (QIAGEN). Polymerase chain reaction (PCR) under standard conditions was used to amplify an 812-bp region that includes the first 807 bp of the cytochrome *b* mitochondrial region. We used the forward primer GluRF2 (Hyde and Vetter 2007), which primes in the tRNA-Glu sequence flanking the gene, and the reverse primer (5′-CGAACAGGAARTATCAYTCTGG-3′) CB3-RF2 (J. Hyde, unpublished data), which primes at the 3′ end of the cytochrome *b* coding region. PCR product was cleaned with a MultiScreen$_{HTS}$ 96-well filtration system (Millipore, Bedford, Massachusetts) and sequenced using ABI Big Dye Terminator version 3. Sequence reactions were purified with CleanSEQ (Agencourt Bioscience, Beverly, Massachusetts) and data was collected with a Prism 3100 Genetic Analyser (Applied Biosystems Incorporated, ABI, Foster City, California) using an 80 cm capillary and POP 4 polymer. Forward and reverse sequences were aligned and edited using Sequencher version 4.6 for Macintosh (Gene Codes Corporation).

Six μsat loci were genotyped on an ABI 3100 using fluorescently labeled PCR primers developed for other rockfish species. We used the following published loci: *Sra*15-8, *Sra*16-5 (Westerman et al. 2005), *Spi*6, *Spi*10, *Spi*4 (Gomez-Uchida et al. 2003), and *Sal*3 (Miller et al. 2000). PCR amplifications were carried out in 10 μL total reaction volumes containing 3.0 mmol/L (*Sra*15-8, *Sra*16-5, *Spi*10, and *Spi*4) or 2 mmol/L (*Spi*6 and *Sal*3) $MgCl_2$ concentration, 1× Flexi PCR buffer, 0.2 mmol/L each dNTPs, 0.2 μmol/L of each primer, 0.25 U Promega *Taq* Polymerase, and approximately 4 ng genomic DNA. PCR products were diluted 1/160 to 1/40 before electrophoresis (500 LIZ *size* standard ABI). Electrophoresis data were analyzed for genotyping by using GeneScan and Genotyper version 3.7 software (Applied Biosystems).

Data analysis

For each of the 21 collections, microchecker version 2.2.3 (Van Oosterhout et al. 2004) was used to test the μsat loci for null alleles and scoring errors due to stuttering and large allele dropout. In addition, for each collection, FSTAT version 2.9.3.2 (Goudet 2002) was used to calculate pairwise F_{ST} and test for Hardy–Weinberg expectation (F_{IS} and F_{IT} calculated according to Weir and Cockerham (1984)) and linkage disequilibrium. Probability (*P*) values for pairwise F_{ST} were obtained by 210 000 permutations of genotypes among collections. FSTAT version 2.9.3.2 (Goudet 2002) was also used to estimate allelic richness (AR), observed heterozygosity (H_o), and expected heterozygosity (H_e). Nei's (1987) unbiased gene diversity (H_e) was averaged over all loci and calculated with standard error in Microsoft Excel Microsatellite Toolkit version 3.1 (Park 2002). We performed a principal component analysis (PCA) on the μsat allele-frequency data by using the program Genetix version 4.03 (Belkhir et al. 2004). We used the μsat data to estimate the number of migrants per generation by the equation $Nm = (1 - F_{ST})/4(F_{ST})$, which assumes an island model (Wright 1969). In addition, we grouped all collections into two populations north and south of Cape Mendocino and estimated *Nm* using the private allele method (Barton and Slatkin 1986) as implemented in Genepop version 4.0 (Raymond and Rousset 1995).

Structure version 2.3.2 (Pritchard et al. 2000) was used to determine how many populations might be represented among our collections. An initial burn-in of 50 000 iterations followed by 500 000 iterations of the Markov Chain Monte-Carlo method was used to generate posterior probabilities. We used the new feature in Structure that takes into account spatial information (i.e., from which collection an individual was sampled) to modify the prior distribution for each individual's population assignment to increase the ability to detect structure at low levels of divergence (Hubisz et al. 2009). We ran 10 iterations of *K* values 1 through 9 and averaged the estimated log-likelihoods [ln(Pr*K*)] to determine the most likely value of *K*. We calculated ΔK using the method described by Evanno et al. (2005). After selection of $K = 2$, 40 iterations were run and the 10 iterations with the highest ln(Pr*K*) were averaged using the *FullSearch* algorithm in CLUMPP (Jakobsson and Rosenberg 2007), which finds the best configuration of those 10 iterations by optimizing pairwise similarity (*H*').

Arlequin version 3.1 (Excoffier et al. 2006) produced *F*-statistics for mtDNA sequence data based on haplotype frequencies, and was used to measure gene and nucleotide diversity, and Tajima's *D*. We performed multidimensional scaling (MDS) on the mtDNA pairwise F_{ST} matrix using the software package SPSS version 11.0.2 (SPSS for Mac OSX, SPSS Inc., Chicago, Illinois). We used the analysis of molecular variance (AMOVA, Excoffier et al. 1992), with Arlequin version 3.1 (Excoffier et al. 2006) to examine hierarchical population structure of all collections separated into two groups (based on results from Structure). Two groups were first defined as (*i*) the most southern collection versus (*ii*) the remaining northern collections. We then transferred nearest neighbor collections, one at a time, into the southern group for subsequent tests until we had performed an AMOVA for every grouping that splits the collections into two groups along their linear latitudinal axis. The F_{CT} values (variance component representing variance between regions) and corresponding *P* values from these 20 AMOVA tests were calculated based on 1000 permutations.

We calculated isolation-by-distance slopes (IBD), using only collections of $n \geq 10$ individuals for both the mtDNA and μsat data. PASSaGE version 1.0 (Rosenberg 2001) was used to generate a geographical distance matrix from the latitude/longitude coordinates of the collections. The pairwise F_{ST} values were transformed to $[F_{ST}/(1 - F_{ST})]$ to account for the linear distribution of the collections (Rousset

Table 1. Collection locations, sample sizes, and basic population genetic statistics for microsatellite data.

Col. No.	Location	Latitude	Longitude	*N*	*A*	AR	H_O	H_E	F_{IS}	*P*
21	Yakutat, AK	59.60000	−140.08000	1	1.667	NA	0.667	0.667	NA	NA
20	Salisbury Sound, AK	57.35284	−135.90911	7	5.000	NA	0.690	0.694	0.006	0.550
19	Iphegenia Bay, AK	55.75613	−133.98350	1	2.000	NA	1.000	1.000	NA	NA
18	Craig, AK	54.76883	−133.78431	45	8.833	5.279	0.615	0.610	−0.009	0.634
17	N. Vancouver Is., BC	50.87500	−129.25000	94	10.500	5.666	0.635	0.649	0.022	0.165
16	S. Vancouver Is., BC	49.12402	−126.67894	85	10.000	5.574	0.669	0.667	−0.003	0.575
15	Neah Bay, WA	48.50554	−125.79765	75	10.167	5.496	0.664	0.653	−0.017	0.775
14	Astoria, OR	46.07500	−124.51761	108	10.167	5.632	0.667	0.676	0.014	0.265
13	Cannon Beach, OR	45.90849	−124.46891	33	8.500	5.761	0.692	0.680	−0.018	0.724
12	Cannon Beach, OR	45.69968	−124.50905	42	8.000	5.154	0.625	0.627	0.003	0.484
11	Florence, OR	43.97590	−124.65957	75	9.167	5.762	0.659	0.676	0.024	0.155
10	Port Orford, OR	42.72806	−124.67289	99	9.667	5.846	0.709	0.700	−0.013	0.727
9	Crescent City, CA	41.75000	−124.08333	2	3.000	NA	0.667	0.778	0.200	0.112
8	Eureka, CA	40.40833	−124.55500	5	4.000	NA	0.633	0.641	0.013	0.541
7	Fort Bragg, CA	39.48122	−123.85387	65	10.000	5.955	0.728	0.716	−0.017	0.770
6	San Francisco, CA	38.07500	−123.45167	97	9.667	5.973	0.747	0.732	−0.020	0.848
5	San Francisco, CA	37.87756	−123.04324	68	9.333	5.775	0.727	0.720	−0.009	0.624
4	Santa Cruz, CA	36.81341	−122.12877	41	8.333	6.003	0.759	0.744	−0.021	0.726
3	San Luis Obispo, CA	35.15105	−120.82881	53	9.500	6.054	0.742	0.749	0.009	0.395
2	Santa Barbara, CA	34.34948	−119.60536	10	5.500	5.500	0.783	0.732	−0.075	0.891
1	San Miguel Is., CA	34.09976	−120.54119	7	5.167	NA	0.786	0.744	−0.062	0.838
Total				1013						

Note: Col., collection; *N*, sample size; A, average number of alleles across loci; AR, allelic richness based on 10 individuals; H_O and H_E, the mean observed and expected heterozygosities, respectively; *P*, proportion of randomizations greater than the observed F_{IS}; AK, Alaska; BC, British Columbia; WA, Washington; OR, Oregon; CA, California.

1997) then regressed against the pairwise geographic distances in Excel. PASSaGE was used to perform basic Mantel tests for comparing the correlation coefficients from a model of IBD gene flow versus a model of a gene-flow barrier. The basic Mantel tests compared a matrix of linearized genetic distance with either a matrix of pairwise geographical distances (IBD model) or a "regional affiliation" matrix of ones and zeros (a "1" or "0" was assigned depending on whether a particular pair of collections was located on the opposite or same side of a putative dispersal barrier, respectively). Partial Mantel tests were performed testing correlations among two of the three matrices (genetic distance, regional affiliation, and geographic distance) while holding the third matrix constant. Significance for these Mantel tests was obtained by random permutation using 9999 iterations.

We performed a nested clade phylogeographical analysis (NCPA) of mtDNA sequences by first constructing a haplotype tree assuming parsimony (Templeton et al. 1992) implemented with the program TCS version 1.21 (Clement et al. 2000). The haplotype tree was used to define a series of hierarchically nested clades (Templeton et al. 1987; Templeton and Sing 1993). Loops in the network were broken according to a set of guidelines based on predictions from coalescence theory (Posada and Crandall 2001). The program GeoDis version 2.6 (Posada et al. 2000) was used to test for significant associations of haplotype genealogy and geographical distance and the Dunn–Šidák correction was applied to correct the significance of the test results for multiple testing (Templeton 2008; 2009*b*). Results were interpreted using the latest version of the GeoDis inference key. The network of yellowtail haplotypes was rooted by using two related species (*S. melanops* and *S. serranoides* Genbank accession No. FJ664347 and FJ664348, respectively). As an alternative to the outgroup species rooting, we calculated outgroup weights by the method of Castelloe and Templeton (1994). This rooting method appears robust when there are large interspecific differences with respect to intraspecific differences. We used the method described by Templeton (2003) to estimate the time in years (*t*) of events inferred by NCPA with the following equation: $t = [D_{xy} - 0.5 \times (D_x + D_y)] \times$ mutation rate, where D_{xy} is the average divergence between the recently derived clade from the nearest older clade, and D_x and D_y are the average sequence diversity within the recent and older clade, respectively. We calculated confidence intervals for the time estimate using a gamma function to account for genetic drift in modeling the distribution of time (Kimura 1970). We used the mutation rate of 0.71% sequence divergence per million years, which was also used by Rocha-Olivares et al. (1999) for cytochrome *b* in fishes.

Results

Basic population genetic analyses

The six μsat loci did not deviate from Hardy–Weinberg expectation for genotype frequencies (Table 1), nor did loci show evidence of linkage disequilibrium. In addition, results from error checking with microchecker yielded no evidence for scoring error due to stuttering or large allele dropout, and no evidence for null alleles for any locus in any of the collections. Both the principal component analysis (PCA) of the μsat dataset (Fig. 2) and the multidimensional scaling plot of the mtDNA dataset (not shown) — using only collections with $n \geq 10$ to minimize sampling variance — showed

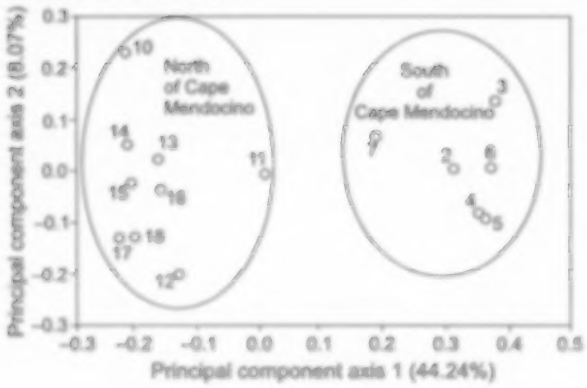


Fig. 2. Principal component analysis using genotypic data from six microsatellite loci. A genetic break is apparent between collections located north and south of Cape Mendocino. This analysis includes only populations with $n \geq 10$.

two main clusters composed of the same collections. Southern collections (2–7) were in one cluster, and the rest of the collections further north (10–18) were in the other.

The Structure results also showed evidence of two genetic groups. The mean ln(PrK) was highest for $K = 2$ [ln(PrK) = −19715, −19410, and −19595 for $K = 1$, 2, and 3 respectively]. The ΔK derivative method also shows $K = 2$ having the highest value ($\Delta K = 30.9$) compared with values (ΔKs < 10) at all other K settings (range of 1–7). Although Structure has been shown to have poor performance when migrants per generation (Nm) is high (>5) and the number of genetic markers is low (Waples and Gaggiotti 2006), we were able to obtain consistent results of individual assignment (Q) values across replicates and agreement between Structure and the ΔK derivative method, despite having relatively weak population differentiation. The better performance of Structure is largely due to the new feature that uses prior information from sampling localities to inform cluster assignment. This feature has been shown to improve accuracy in cluster assignment and selection of the correct value of K, even with datasets that have low numbers of loci (<10) and weak population differentiation (Hubisz et al. 2009).

To obtain a set of robust individual assignment (Q) values to $K = 2$ clusters, the *FullSearch* algorithm in CLUMPP was used to find the optimal alignment of clusters across multiple runs. The total number of all possible alignment configurations is computed by the equation $(K!)^{R-1}$. In our case, there were two K clusters and 10 replicates (R), which result in a total of 512 possible configurations. After clusters were aligned (final optimized pairwise similarity $H' = 0.979$), CLUMPP was used to average individual Q values across the 10 Structure replicates at $K = 2$. The proportion of assignment of individuals in collections to the two clusters identified by Structure was strongly associated with geographical location, north and south of Cape Mendocino (Figure 3). In fact, no individuals from the northern region assigned to the southern cluster (with individual $Q > 75\%$), and only four individuals from the southern region assigned to the northern cluster (with individual $Q > 75\%$). These four individuals were all found in collection 7, and only accounted for 6% of the total individuals in the collection. Collections 8 and 9, located closest south and north of Cape Mendocino, respectively, have insufficient size to make any strong inferences. However, Structure results showed evidence that this Cape Mendocino region may be located near the most dramatic transition. Collection 8 had three individuals out of five with high assignment (>75%) to the southern cluster, and the other two had assignments of 56% and 71% to the southern cluster. Whereas, collection 9 had one individual that assigned to the northern cluster (89%) and one that assigned to the southern (72%).

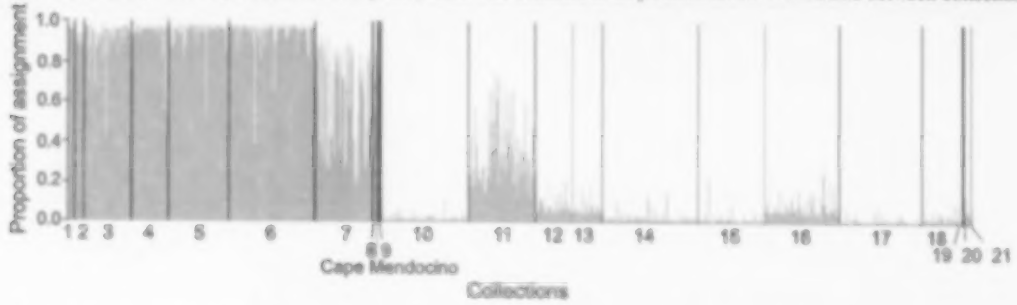
Cytonuclear disequilibrium, which is a nonrandom association of genotypes at nuclear loci with haplotypes at mtDNA or other cytoplasmically inherited organellar DNA, can occur as a result of strong assortative mating in a recently hybridized population (Asmussen et al. 1987). Evidence for this disequilibrium would be indicated if Structure assignment were correlated with mtDNA haplotypes. However, based on the following results, we did not find evidence for this pattern. Of the four migrants in collection 7 that assigned strongly to the northern cluster (Q >75%), three of them had mtDNA haplotype B (the more southerly distributed haplotype), and only one had haplotype A (the more northerly distributed haplotype). In addition, Structure cluster assignments were not significantly different for individuals that had mtDNA haplotype A compared with those with haplotype B within collections 7 and 10, which are nearest to the transitional zone of genetic exchange at Cape Mendocino. Average assignment to the southern Structure cluster for A- and B-haplotype individuals in collection 7 was 47.6% was 62.9%, respectively (analysis of variance, ANOVA; $P = 0.07$). In collection 10, average assignment to the northern cluster for A-haplotype and B-haplotype individuals was 98.0% and 97.7%, respectively (ANOVA; $P = 0.54$).

The matrices of the pairwise F_{ST} values for both mtDNA and μsat datasets are provided as supplemental materials (see supplementary data,[2] Table S1). In general, the significant pairwise F_{ST} values correspond with the existence of two genetically divergent groups in agreement with results from Structure and the MDS and PCA plots showing two clusters of collections. For μsats, we found an overall F_{ST} value of 0.011 with 95% confidence interval of 0.008–0.015. These values were the same even when only collections with 10 or more individuals were considered.

The analyses presented above have indicated a genetic break somewhere between collections 7 and 10, however, to examine this genetic break location statistically, we used all 21 collections and calculated F_{CT} values from AMOVAs (Table 2). Both the mtDNA and μsat datasets showed that the largest significant F_{CT} values were obtained with a division between collections 9 and 8, north and south of Cape Mendocino. AMOVA results for this particular grouping yielded an overall F_{ST} of 0.32 ($P \ll 0.001$) and overall F_{ST} of 0.02 ($P \ll 0.001$) for mtDNA and μsat, respectively. The largest component of genetic variation was observed within collections (67.88% and 97.8% for mtDNA and μsat, respectively). The portion of variation captured between the two

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

Fig. 3. Individual assignment values from the Structure analysis using microsatellite data and a cluster setting of $K = 2$. The collections are depicted on the x axis and posterior probabilities along the y axis. The location of Cape Mendocino is indicated between collections 8 and 9.



groups north and south of Cape Mendocino between collections 9 and 8 is $F_{CT} = 0.32$ ($P \ll 0.001$) and $F_{CT} = 0.02$ ($P \ll 0.001$) for mtDNA and μsat, respectively. The remaining variation is not significant among collections within groups ($F_{SC} = 0.01$, $P = 0.06$) for mtDNA, but shows minimal significance for μsat ($F_{SC} = 0.001$, $P = 0.03$). Using the equation $Nm = (1 - F_{ST})/4(F_{ST})$ (Wright 1969), and the value of F_{ST} estimated between regions separated by Cape Mendocino ($F_{ST} = 0.02$), the number of effective migrants exchanged across regions per generation is $Nm = 12.2$. The private allele method (Barton and Slatkin 1986) produced a similar estimate: $Nm = 9.6$.

Genetic diversity north and south of Cape Mendocino

Separation of the collections into two regions at Cape Mendocino reveals significantly greater gene diversity in the south compared with the north (Table 3). Moreover, the regression of Nei's unbiased gene diversity (averaged across all six loci) and latitude was found to be significant and negatively correlated (Figure 4). When loci were examined individually, significant negative correlations of gene diversity and latitude were observed for four loci *Sra*15-8, *Sra*16-5, *Spi*4, and *Sal*3 ($R^2 = 0.77$, 0.58, 0.82, and 0.34, respectively, P values <0.05). The remaining two loci (*Spi*6 and *Spi*10), were not significantly correlated.

The trend in values of these μsat diversity measures was reversed relative to mtDNA haplotype diversity (Table 2). MtDNA haplotype diversity increased with increasing latitude (significant trend; $P < 0.001$). Tajima's D values were mostly significantly negative among the collections ($n > 10$) south of Cape Mendocino.

Contemporary dispersal

At the range-wide level, regression analysis of genetic distance as a function of geographic distance demonstrated significant isolation by distance (IBD) for both DNA datasets (Figure 5). The mtDNA data set showed a slope of 0.2228 for linearized F_{ST} per 1000 km ($r^2 = 0.24$, $P \ll 0.001$). For the μsat dataset, the slope equates to 0.0167 linearized F_{ST} per 1000 km ($r^2 = 0.62$, $P \ll 0.001$). Inclusion of collections on opposite sides of Cape Mendocino inflated IBD estimates. To remove this Cape Mendocino bias from our IBD signal, we tested IBD over the species ranges on either side of Cape Mendocino. For the mtDNA data, there was no significant IBD either north or south of Cape Mendocino. However, for the μsat data, the northern collections continued to exhibit significant IBD albeit lower than the total range: 0.0044 linearized F_{ST} per 1000 km ($r^2 = 0.25$, $P < 0.01$). The southern collections did not exhibit a significant positive regression for a single-tailed test: –0.011 linearized F_{ST} per 1000 km ($r^2 = 0.28$, $P = 0.98$).

We found that for both mtDNA and μsat datasets, the barrier Mantel test yielded a higher correlation than the IBD Mantel test (Table 4). In partial Mantel tests holding regional affiliation constant, the μsat IBD test was still significant, but the mtDNA correlation was not. Thus, IBD gene flow may have some influence on the genetic structure; however, the barrier gene-flow model was the best overall fit on the regional level (thousands of kilometres).

Phylogeographical analyses

We observed 47 haplotypes in yellowtail rockfish (GenBank accession No. FJ664349–FJ664395) that were used to construct a parsimony network (Fig. 6). To perform nested clade phylogeographical analysis (NCPA), we broke loops in the network (breakpoints indicated by cross marks (×) in Fig. 6) to produce the nested network shown in Fig. 1. However, 16 alternative nested networks were also considered and produced similar inferences.

The first significant NCPA inference involved clade 1-8 nested within clade 2-2 (Table 5). This pattern indicated a contiguous range expansion. Clade 1-8 has a geographic center point that was located further north compared with the entire 2-2 nesting clade (see supplementary data,[2] Table S2), and so the expansion occurred from the more southerly ancestral haplotype distribution of the nesting clade into the north.

The second significant inference occurred at the total cladogram level and the outcome depended on whether clade 2-1 or 2-2 was determined to be ancestral. When we used both olive and black rockfish as outgroup species, we observed connections to both clade 2-1 and clade 2-2, although clade 2-1 was more parsimonious by a single base substitution (Fig. 6). If clade 2-1 were ancestral, the inference at the total cladogram level would be isolation by distance. However, a more reliable rooting of the network obtained using outgroup probabilities (see details in methods), consistently indicated clade 2-2 has the highest outgroup probability (71.4%) versus clade 2-1 (19.3%). Even in the case when alternative nesting collapses haplotypes A and B in the same two-step clade, haplotype B has the highest outgroup probability value. This more reliable approach changes the total cladogram inference to contiguous range expansion.

Table 2. Basic mtDNA diversity measures by collection site.

Col. No.	*N*	No. of haplotypes	Mean pairwise differences ±SD	No. of segregating sites (*S*) diversity	Haplotype diversity (*h*) ±SD	Nucleotide diversity (π)	Tajima's *D*		Col. No.[a]	Results of AMOVA	
							D	*P*		mtDNA F_{CT}	µsat F_{CT}
21	1	1	0.000±0.000	0	1.000±0.000	0.0000±0.0000	0.000	1.000	21		
20	7	4	1.048±0.785	3	0.714±0.181	0.0013±0.0011	–0.654	0.317	20	–0.1804	–0.0033
19	1	1	0.000±0.000	0	1.000±0.000	0.0000±0.0000	0.000	1.000	19	0.0112	–0.0154
18	45	5	1.089±0.729	4	0.663±0.060	0.0013±0.0010	0.442	0.671	18	0.0439	–0.0115
17	95	8	0.944±0.653	7	0.640±0.042	0.0011±0.0009	–0.729	0.287	17	–0.0501	0.0043
16	85	7	0.757±0.563	6	0.517±0.057	0.0009±0.0007	–0.848	0.247	16	–0.0142	0.0050
15	73	11	1.015±0.689	10	0.666±0.046	0.0012±0.0009	–1.358	0.062	15	0.0365	0.0040
14	108	10	0.786±0.576	9	0.533±0.050	0.0009±0.0008	–1.336	0.076	14	0.0511	**0.0057**
13	32	6	0.970±0.677	5	0.669±0.072	0.0012±0.0009	–0.588	0.305	13	**0.1122**	**0.0088**
12	42	5	0.891±0.635	4	0.664±0.045	0.0011±0.0008	–0.098	0.488	12	**0.1252**	**0.0096**
11	74	9	0.947±0.657	11	0.628±0.042	0.0011±0.0009	–1.583	0.027	11	**0.1319**	**0.0117**
10	98	8	0.755±0.561	10	0.520±0.052	0.0009±0.0007	–1.566	0.036	10	**0.1669**	**0.0130**
9	2	2	1.000±1.000	1	1.000±0.500	0.0012±0.0017	0.000	1.000	9	**0.3142**	**0.0207**
Cape Mendocino											
8	5	3	0.800±0.681	2	0.700±0.218	0.0010±0.0010	–0.973	0.202	8	**0.3160**	**0.0208**
7	65	10	0.636±0.503	10	0.525±0.070	0.0008±0.0007	–1.909	0.009	7	**0.3099**	**0.0205**
6	96	13	0.470±0.414	12	0.388±0.064	0.0006±0.0006	–2.133	0.003	6	**0.2809**	**0.0201**
5	67	9	0.564±0.465	11	0.429±0.071	0.0007±0.0006	–2.094	0.002	5	**0.2064**	**0.0155**
4	40	9	0.679±0.530	9	0.540±0.092	0.0008±0.0007	–1.984	0.007	4	**0.1620**	**0.0128**
3	53	6	0.448±0.405	5	0.413±0.078	0.0005±0.0005	–1.420	0.052	3	**0.1348**	**0.0126**
2	10	3	0.400±0.403	2	0.378±0.181	0.0005±0.0005	–1.401	0.078	2	0.0341	0.0121
1	7	2	0.286±0.341	1	0.286±0.196	0.0003±0.0005	–1.006	0.234	1	–0.0670	0.0323
Total	1006										

Note: *N*, sample size; µsat, microsatellite. Sample locations as in Table 1. At the far right are the F_{CT} values for AMOVAs performed on heuristic groupings of collections. Bolded and shaded F_{CT} values indicate significance at the 0.05 and 0.001 alpha levels, respectively.

[a]The 21 collections were systematically divided into two groups, and the corresponding F_{CT} value for each division is listed to the right of the collection located on the southern side of the division, e.g., when collections were split between collections 8 and 9, the F_{CT} values were highest and equated to 0.3160 and 0.0208 for mtDNA and µsat datasets, respectively.

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Table 3. Genetic diversity comparisons between regions north and south of Cape Mendocino, based on microsatellite data.

Locus	Region	Allelic richness	H_o	H_s
*Sru*15–8	North	**4.707**	**0.505**	**0.516**
	South	**5.714**	**0.640**	**0.649**
*Sru*16–5	North	4.575	**0.585**	**0.586**
	South	5.026	**0.704**	**0.681**
*Spi*6	North	7.482	0.846	0.849
	South	6.906	0.847	0.841
*Spi*10	North	6.264	0.820	0.818
	South	6.431	0.849	0.813
*Spi*4	North	**4.672**	**0.455**	**0.450**
	South	**5.448**	**0.599**	**0.621**
*Sal*3	North	5.745	0.760	0.761
	South	5.736	0.808	0.780
Total	North	**5.574**	**0.662**	**0.663**
	South	**5.877**	**0.741**	**0.731**

Note: Values in bold show significant differences ($P < 0.05$).

Using a sequence divergence value of 0.71% per million years, the range expansion inferences from NCPA at the two-step clade and total cladogram levels yielded 95% confidence intervals that failed to reject that these events may have coincided with last glacial maximum (LGM) (22 000 to 17 000 bp). The estimated timing of these two events were 168 thousand years before present (kybp; 6000–597 000, 95% C.I.) and 174 kybp (7000– 603 000, 95% C.I.) for the 2-step clade and the total cladogram range expansions, respectively.

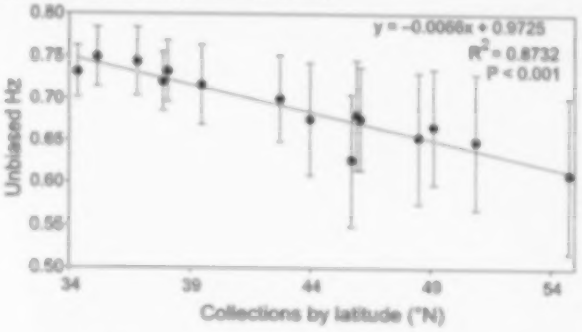
Discussion

General population genetic patterns in yellowtail rockfish

Our study is the first to report the presence of a steep genetic cline in yellowtail rockfish. We found geographic concordance between the mtDNA and μsat datasets, which both showed large genetic divergence between two groups of collections located north and south of Cape Mendocino, California. Concordance across molecular markers taken together with the patterns of varying degrees of genetic divergence in other marine species, including two rockfish species, *Sebastes mystinus* (Cope 2004; Burford and Bernardi 2008; Burford 2009) and *Sebastes miniatus* (Hyde and Vetter 2009), supports that coastal provinces north and south of the Cape Mendocino region experience different evolutionary trajectories.

At this time, we are unable to attribute a single mechanism that operates to influence differences within and among species that inhabit the northern and southern provinces separated near the Cape Mendocino region. However, there are now three species of rockfish that show differences to varying degrees of isolation between these regions, and together with additional rockfish species could be studied for further insight into the common evolutionary processes influencing these patterns. On one extreme is the example of reproductively isolated genetic lineages of blue rockfish, whose distributions were observed to broadly overlap in a highly variable step-cline between southern Oregon and northern California (Burford 2009). Nearing the opposite extreme is the example of vermilion rockfish in which a minor genetic discontinuity (F_{CT} of ~0.01) centered on Cape Mendocino was observed with cytochrome *b* sequence data after taking into account a major break (F_{CT} ~0.14) at Point Conception (Hyde and Vetter 2009). Yellowtail rockfish is an intermediate example of genetic isolation centered on the Cape Mendocino region, because it showed greater regional genetic divergence (F_{CT} 0.32, 0.02 for mtDNA and μsat, respectively) compared with the vermilion rockfish, but no evidence of complete reproductive isolation (i.e., no Hardy–Weinberg deviation of μsat loci in any transitional collection and lack of reciprocal monophyly based on mtDNA). We suggest the primary mechanism responsible for genetic divergence observed among populations of yellowtail rockfishes is a contemporary process that either limits the rate of larval dispersal by means of a discrete barrier within the Cape Mendocino region or by means of differences in environmental factors that characterize the provinces on either side of the Cape Mendocino region (limiting success of recruitment exchange through selection). In addition to contemporary processes limiting exchange, we hypothesize that secondary mechanisms involving historical events may also be responsible for genetic divergence. Following discussions of these primary and secondary mechanisms, we describe potential management implications of our conclusions.

Fig. 4. Correlation of Nei's unbiased gene diversity (H_s) averaged across six microsatellite loci with latitude. This analysis includes only populations with $n \geq 10$. Error bars are based on standard error as calculated in Microsatellite Toolkit.



Contemporary mechanisms

Four larval dispersal models were discussed in Gunderson and Vetter (2006) as a way to explain the range of genetic patterns observed among North Pacific rocky reef fishes. These models are broad dispersal, mesoscale dispersal, diffusive dispersal, and nondispersing. The differences in the models relate to the way in which a population of a particular species receives larval recruits. Broad dispersal and nondispersing models are at the two extremes in that the former is characterized by a set of populations that recruit from a common pool of larvae, whereas the latter is characterized by a network of closed, self-recruiting populations. Yellowtail rockfish appears to be intermediate in relation to these extremes, and therefore, may be better described by either the mesoscale or diffusive dispersal models. In the mesoscale model, populations recruit from regional pools of lar-

Fig. 5. Isolation by distance F_{ST} plots for mtDNA (*a*) and microsatellites (*b*). Each square or circle on the plots represents a pairwise F_{ST} value for a comparison of two collections. Filled squares represent pairwise F_{ST} values between collections on opposite sides of Cape Mendocino. Open circles represent all other F_{ST} comparisons between collections on the same side of Cape Mendocino. The plot for microsatellite data (*b*) also shows grey squares representing the pairwise F_{ST} values only on the northern side of Cape Mendocino. The equations at the top of both plots refer to the regressions using all pairwise comparisons. The second equation at the lower portion of the microsatellite plot (*b*) refers to the regression of only the pairwise comparisons on the northern side of Cape Mendocino.

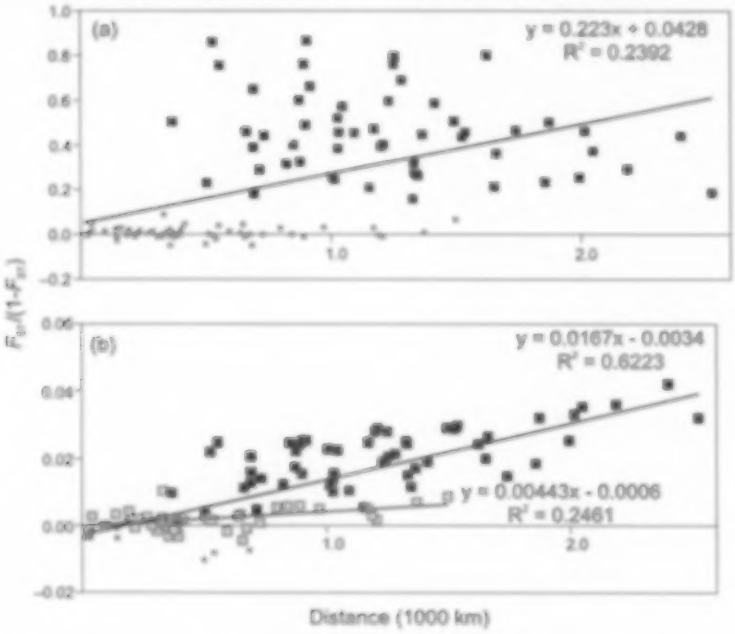


Table 4. Mantel correlations (*r*) for pairwise F_{ST} matrices with geographic distances (Geodist) and geographic region (Region) for microsatellite and mitochondrial data.

		Microsatellites		Mitochondrial DNA	
Mantel	Matrices	*r*	*P*	*r*	*P*
Basic	PW F_{ST} × Geodist	0.7888	0.0001	0.489	0.0011
	PW F_{ST} × Region	0.8408	0.0001	0.8567	0.0006
Partial	PW F_{ST} × Geodist; Region	0.5773	0.0001	-0.1933	0.8585
	PW F_{ST} × Region; Geodist	0.6948	0.0001	0.8144	0.0001

Notes: *P* values are the number of 9999 iterations that were greater than the observed *r*. In the partial Mantel tests, the first two terms were tested for a correlation while the third term (separated by a semicolon) was held constant.

vae and in the diffusive dispersal model, populations are primarily self-recruiting but receive some recruits from adjacent populations in a stepping stone manner. Following the guidelines stated by Johansson et al. (2008), these two models can be distinguished by comparing isolation by distance (IBD) regressions at both the coast-wide level (thousands of kilometres) and at finer scales (hundreds of kilometres). The diffusive model is predicted to show significant IBD at both spatial scales; whereas meso-scale dispersal is predicted to show significant IBD only at the coast-wide level. In addition, we suggest that, under a diffusive dispersal model, the correlation coefficient (*r*) of genetic distance versus geographic distance at the coast-wide level should be higher than the correlation of a genetic distance versus regional affiliation as measured by basic Mantel tests. The reverse should be true for mesoscale dispersal at the coast-wide level. Furthermore, under a diffusive dispersal model, in partial Mantel tests when regional affiliation is held constant, genetic distance versus geographic distance should continue to generate high *r* values.

We find significant IBD regressions at our coast-wide scale, but at fine spatial scales (i.e., within regions north and south of Cape Mendocino) IBD regressions weaken (μsats in the northern region) or disappear (μsat in the southern region and mtDNA in both northern and southern regions). In basic Mantel tests, both mtDNA and μsat correlation coefficients are higher for a regional affiliation than for geographic distance. In partial Mantel tests holding regional affiliation constant, the μsat correlation of geographic distance versus genetic distance was still significant but the mtDNA correlation was not. Therefore, we conclude that yellowtail rockfish population structure may best be de-

Fig. 6. An mtDNA haplotype network constructed using maximum parsimony in the TCS program. Each line represents a single mutation. Open circles represent observed haplotypes and filled circles represent unobserved haplotypes. Within each haplotype the notation indicates the number of samples (omitted for singletons) followed by a period and the alphanumeric designation of the haplotype (haplotype names correspond with those in in the supplementary data,[2] Table S2). The cross marks (×) shown on loops indicate which connections were broken to obtain the nesting in Fig. 1.

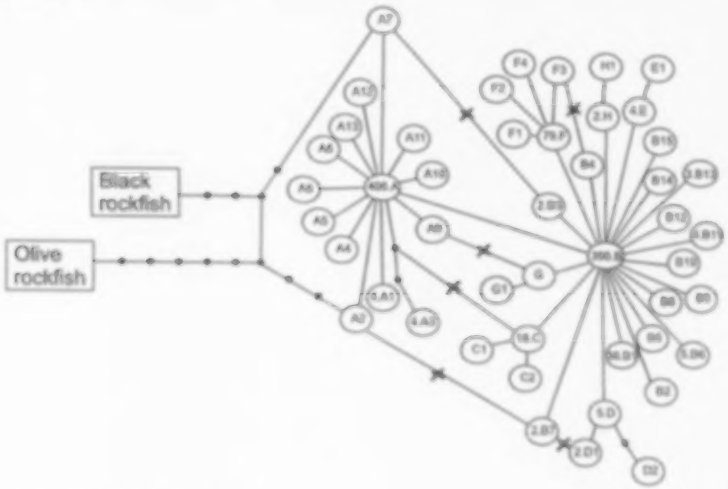


Table 5. Nested clade phylogeographical analysis results.

	Outgroup			
Clade	Prob.	χ^2 statistic	*P*	Chain of inference
1-1	0.193	183.634	0.421	
1-2	0.000	—	—	
1-3	0.233	234.693	0.567	
1-4	0.096	24.167	0.361	
1-5	0.093	7.000	0.153	
1-6	0.000	—	—	
1-7	0.092	5.000	0.601	
1-8	0.111	23.299	0.824	
1-9	0.091	2.000	1.000	
1-10	0.092	0.750	1.000	
2-1	0.193	12.401	0.293	
2-2	0.714	213.340	0.018*	1 - 2 - 11 - 12[a] No. Contiguous range expansion
2-3	0.093	8.000	0.510	
Total cladogram 1	NA	347.847	0.000***	1 - 2 - 3 - 4[b] No. Restricted gene flow with isolation by distance
Total cladogram 2	NA	347.847	0.000***	1 - 2 - 11 - 12[c] No. Contiguous range expansion

Note: Clade numbers correspond to the nested clade levels in Fig. 1. Prob., probability; —, no data; *, $P < 0.05$; ***, $P < 0.01$. The outgroup probabilities were most important in designating whether clade 2-1 or clade 2-2 was more likely to be the outgroup clade (see Results for explanation). Only significant ($P < 0.05$) *P* values for χ^2 statistics were used to make a phylogeographical inference according to the NCPA key. Two inferences are given at the total cladogram level depending upon which two-step clade (2-1 or 2-2) is the outgroup.

[a]This inference refers to the 1-step clades within clade 2-2.

[b]This inference refers to all 2-step clades within the total cladogram in which clade 2-1 is the most ancestral clade, i.e. the outgroup clade (alternative 1).

[c]This inference refers to all 2-step clades within the total cladogram in which clade 2-2 is the most ancestral clade (alternative 2).

scribed by the mesoscale model, but also cannot rule out that diffusive dispersal is operating within regions north of Cape Mendocino. This result is important because even though the regressions of IBD for our genetic datasets were significant, IBD dispersal alone does not best explain the population structure observed in this species.

Aside from our genetic study, other evidence supports demographic independence of the regions separated by Cape Mendocino. Vermilion rockfish also have a genetic barrier in the vicinity of Cape Mendocino, as well as other regions including Point Conception (Hyde and Vetter 2009). At least one marine invertebrate species (acorn barnacle, *Balanus glandula*, Sotka et al. 2004) implicated the Cape Mendocino region and a second invertebrate study emphasized the region between two promontories Cape Blanco and Cape Mendocino as a putative barrier (mud crab, *Hemigrapsus oregonensis*, Petersen 2007). The region near Cape Mendocino has also been correlated with start/end points of

species' range distributions (Williams and Ralston 2002) and where fish species assemblage structure has been found to shift (Tolimieri and Levin 2006). In addition, Field and Ralston (2005) reported a difference in spatial variability of year class strength between populations of yellowtail, widow, and chilipepper rockfishes north and south of Cape Mendocino. They found that collections south of Cape Mendocino were uncorrelated in yellowtail rockfish recruitment with collections north of Cape Mendocino. In contrast, there was relatively high correlation in yellowtail recruitment among collections within the region north of Cape Mendocino (Field and Ralston 2005). These results are consistent with the mesoscale type of larval dispersal model that we have proposed, and demographic independence of northern and southern stocks.

There are two principal ways that gene exchange may be limited across the Cape Mendocino region, either as a result of a discrete dispersal barrier or as a result of the habitat differences characterizing the coastal provinces separated by this region. Capes or promontories along the west coast are sites of major, upwelling, quasi-permanent eddies and this is particularly true at Cape Mendocino (Magnell et al. 1990; Marchesiello et al. 2003). These currents could advect pelagic larval and juvenile stages offshore, assuming their swimming ability is largely passive (Parrish et al. 1981). The degree to which currents influence dispersal would depend on the strength of this assumption, which has been challenged by a study demonstrating the ability of slow-swimming bivalve larvae to avoid offshore currents (Shanks and Brink 2005). In addition, yellowtail and other rockfishes with winter parturition may be less susceptible to the limiting effects of upwelling on connectivity, because the larval period ends and juveniles have settled before maximal upwelling begins during spring and summer (Laidig et al. 2007). However, there is some evidence that even weaker currents during winter upwelling may be strong enough to influence juvenile rockfish survival and dispersal. One study indicated persistent winter upwelling or downwelling events led to fewer rockfish juveniles in late spring by comparison with juvenile abundance in years with more mild winter upwelling (Ainley et al. 1993).

Species we would expect to be most affected by the putative oceanographic barriers imposed by Cape Mendocino are those characterized by significant population sizes on either side of this location, significant proportion of a pelagic larval/juvenile phase that overlaps with periods of maximal spring/summer upwelling, and adult distributions that extend into deeper waters away from nearshore habitat. The nearshore habitat is thought to reduce the influence of advection on larvae and juveniles (Buonaccorsi et al. 2002). We previously discussed ways in which three species of rockfishes may be influenced by the Cape Mendocino region, but these species (vermilion, blue, and yellowtail rockfishes) do not meet all these expectations. Although the adults of all three species occupy deeper waters than nearshore habitat (Love et al. 2002), their timing of peak parturition occurs in fall (vermilion rockfish; Hyde and Vetter 2009) and winter (blue and yellowtail rockfishes; Love et al. 2002). Additional species will have to be studied at fine geographic spatial scales and it is necessary that they represent a greater range of life-history traits for the influence of this putative barrier to be examined more thoroughly.

Aside from oceanographic forces, there is also a dramatic decrease in rocky habitat shallower than 200 m, which could also act as a discrete barrier in the Cape Mendocino region (Williams and Ralston 2002). Reduction of habitat in this depth range would directly affect adult and juvenile abundance, because it includes depths where yellowtail are commonly found (Love et al. 2002). The decreased availability of habitat could limit the success of juveniles recruited from the regions north and south of this point, serving as a barrier to migration between the populations.

A second way that gene exchange may be limited across the Cape Mendocino region is a product of the habitat differences characterizing the coastal provinces separated by this region. Longhurst (2007) described two ecological compartments distinguished by upwelling currents and biochemical features that correspond to the regions separated by Cape Mendocino. A population adapted to the distinguishing characteristics of one compartment (e.g., through larval behavior and slight differences in timing of parturition) could be maladapted to the other. This isolating mechanism would maintain genetic divergence through natural selection. In this study, we employed presumably neutral markers that are not likely to be the target of selection or even closely linked to gene targets of selection. Rather, these neutral markers should represent background levels of neutral genomic divergence. Mating preference or ecological advantage between these ecological compartments may reduce successful gene flow on a genome-wide level, but particularly at gene regions underpinning traits under selection.

The magnitude of the genetic divergence correlated with Cape Mendocino may also have been influenced by a period of isolation or by a founder event during range expansion. Sotka et al. (2004) suggest it is not likely that a dispersal barrier alone could account for the magnitude of the genetic cline observed in the acorn barnacle, but the contemporary barrier acts to maintain genetic differences that accrued during a period of isolation. Our analyses and comparison of the genetic diversity between the northern and southern regions of yellowtail rockfish coastal range also suggest a strong influence of historical processes.

Earth history events and phylogeography

In rockfishes and other marine species that have protracted pelagic larval stages, historical processes are assumed to be difficult to detect because relatively high rates of dispersal can quickly return populations to a state of equilibrium (e.g., model of decay of genetic divergence from founder effect in copper rockfish; Buonaccorsi et al. 2002; or a model of decay of a genetic break in acorn barnacle; Sotka et al. 2004). Still, several authors have acknowledged that both contemporary and historical processes might have been responsible for the genetic patterns they observed in other rockfish studies (Rocha-Olivares and Vetter 1999; Buonaccorsi et al. 2002; Buonaccorsi et al. 2005). Our phylogeographical analyses of yellowtail rockfish provided two main lines of evidence that support northern range expansion in yellowtail rockfish and may help explain some of the genetic divergence observed between regions north and south of Cape Mendocino. First, the nested clade phylogeo-

graphical analysis (NCPA) results indicated northward range expansions at both the two-step and the total cladogram nesting levels. Second, heterozygosity at a majority of the μsat-loci was negatively correlated with latitude, and the region north of Cape Mendocino was significantly lower in μsat-gene diversity compared with the south. Range expansion can generate genetic structure in which genetic diversity (as measured, e.g., with heterozygosity for μsat loci) declines in the region that experienced invasion (trend observed in cactus; Nason et al. 2002). Northern range expansion would be an expected result of post glacial recolonization as northern coastal habitats became more suitable following the last glacial maximum (LGM) (22 to 17 kybp) (Mann and Hamilton 1995). However, the molecular clock timing estimates of the two main range expansions both had broad 95% confidence intervals,,so we cannot rule out previous or subsequent events to the LGM. Finally, additional support for range expansion may be represented by the large portion of collections showing significant negative Tajima's *D* values in the southern range, which suggests demographic expansion has occurred. This result may be due to an expansion event that has originated from the southern populations as indicated by the NCPA. Clearly demographic changes have affected the northern and southern ranges in different ways and could have contributed to the magnitude of genetic divergence between regions.

Applying genetic results to fisheries management

The PFMC currently manages the US fishery of yellowtail rockfish as two stock units north and south of Cape Mendocino (40°30′N), and we have demonstrated genetic support for maintaining these delineations. Stock delineation at Cape Mendocino was partly determined by the large differences in catch composition of this species north and south of this biogeographic boundary. The northern stock has a higher acceptable biological catch (ABC), which is based on total spawning abundance estimates (ABC is 4320 metric tons (mt) for the northern stock versus 116 mt for the southern) (Wallace and Lai 2005). Although historical events may have contributed to the magnitude of genetic divergence between these stocks, it is likely that contemporary processes are a central process responsible for maintaining this genetic pattern. The isolating mechanisms causing species to experience two different evolutionary trajectories on either side of Cape Mendocino have implications not only for the management of yellowtail but also other rockfishes, other marine fishes, and invertebrates. Low abundance, warming temperatures, and isolation from the northern range are factors that increase risk of extirpation of the southern stock. Our estimation of *Nm* (9.6–12.2) reinforces the notion that emigration from the northern stock could not be expected to buffer that risk on a time scale that is relevant to fishery managers.

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Population differences in otolith chemistry have a genetic basis in *Menidia menidia*

Lora M. Clarke, Simon R. Thorrold, and David O. Conover

Abstract: The number of studies using otolith chemistry as a tool to reconstruct the environmental history of fishes and to detect population structure continues to rise, despite the fact that factors influencing otolith deposition are not fully understood. Many studies have examined the influence of environmental parameters on otolith composition, but none to date have tested the possible influence of intrinsic factors. Using lab broodstock populations, we examined the influence of genetics and temperature on Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca concentrations and partition coefficients in the otoliths of juvenile Atlantic silversides, *Menidia menidia*. Fish from two populations, South Carolina, USA, and Nova Scotia, Canada, were reared in 15, 21, and 27 °C. We found significant ($p < 0.05$) differences in otolith Mg:Ca, Mn:Ca, and Ba:Ca ratios as well as in Mg, Mn, and Ba partition coefficients among populations. Such genetic influences on otolith elemental concentrations have important implications for understanding the physiological mechanisms underlying otolith deposition and enhance the utility of otolith chemistry as a marker of fish population structure in the wild.

Résumé : Le nombre d'études qui utilisent la chimie des otolithes pour reconstituer l'histoire environnementale des poissons et détecter leur structure démographique ne cesse de croître, même si les facteurs qui influencent la déposition dans les otolithes ne sont pas complètement compris. Plusieurs travaux ont examiné l'influence des variables du milieu sur la composition des otolithes, mais aucun n'a à ce jour vérifié l'influence possible des facteurs intrinsèques. En utilisant des populations de reproducteurs de laboratoire, nous examinons l'influence de la génétique et de la température sur les concentrations de Mg:Ca, Mn:Ca, Sr:Ca et Ba:Ca et les coefficients de partitionnement dans les otolithes chez de jeunes capucettes, *Menidia menidia*. Nous avons élevé des poissons de deux populations, de Caroline du Sud, É.-U., et de Nouvelle-Écosse, Canada, à 15, 21 et 27 °C. Il existe des différences significatives ($p < 0.05$) dans les rapports Mg:Ca, Mn:Ca et Ba:Ca et les coefficients de partitionnement de Mg, Mn, et Ba entre les populations. De telles influences génétiques sur les concentrations des éléments dans les otolithes entraînent des conséquences significatives pour la compréhension des mécanismes physiologiques qui sous-tendent la déposition dans les otolithes et augmentent l'utilité de la chimie des otolithes comme marqueur de la structure des populations de poissons en nature.

[Traduit par la Rédaction]

Introduction

The use of otolith chemistry as a tool to reconstruct the environmental history of fishes continues despite the fact that factors influencing otolith deposition are not fully understood. Otoliths are useful tools largely because of the manner in which they are formed. Otolith growth is continual throughout the life of a fish, and new layers are deposited in periodic increments, which are often detectable as daily or annual rings. This characteristic allows otoliths to be useful chronometers and provides a means for determining age and growth of the fish. Additionally, as the new otolith layers are deposited, elements and stable isotopes are incorporated into the calcium carbonate structure creating a chemical signature of ambient environmental conditions (Thresher 1999). Chemical signatures are permanent because otoliths are acellular and metabolically inert and therefore not subject to resorption.

Most studies have assumed that environmental and physiological factors generate differences in otolith chemistry among groups of fish. However, the possibility of intrinsic differences among populations or individuals in regards to elemental deposition is rarely considered. Some authors have speculated that intrinsic factors were responsible for observed differences in otolith chemistry. For instance, Thresher et al. (1994) found chemical differences in the otolith composition of *Nemadactylus macropterus* from various locations and suggested that these differences may have had a genetic component. Other studies have reported individual differences in otoliths within treatments that were likely due

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L.M. Clarke[1,2] **and D.O. Conover.**[3] School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794-5000, USA.
S.R. Thorrold. Biology Department, MS #50, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.

[1]Corresponding author (e-mail: Lora.Clarke@noaa.gov).
[2]Present address: NOAA Fisheries, 1315 East West Highway, Room 15728, Silver Spring, MD 20910, USA.
[3]Present address: Division of Ocean Sciences, National Science Foundation, 4201 Wilson Blvd., Arlington, VA 22230, USA.

to endogenous factors (Fowler et al. 1995). The presence of genotype differences would have important implications for studies using otolith chemistry to reconstruct environmental histories of fishes (Elsdon et al. 2008). To date, however, little is known about the possible influence of genetics on otolith chemistry, and these factors have not been investigated in a controlled laboratory setting.

Environmental and physiological factors, including temperature (e.g., Martin and Wuenschel 2006), water chemistry (e.g., Bath et al. 2000), diet (e.g., Buckel et al. 2004), growth (e.g., Sadovy and Severin 1992), and maturation (e.g., Kalish 1989), are known to influence the chemical composition of fish otoliths, but whether these factors interact with genetic differences is not known. Temperature has been the most widely studied environmental factor, but investigations into the relationship between temperature and elemental deposition in otoliths have led to less than clear results (see review in Campana and Thorrold 2001). The vast majority of studies have focused on strontium, which has been reported to reflect temperature changes in some species during specific life stages (Bath et al. 2000; Elsdon and Gillanders 2002; Martin and Wuenschel 2006), but has not shown a temperature relationship in others (Gallahar and Kingsford 1996; Chesney et al. 1998). Studies involving other elements have produced similar results. Magnesium, manganese, and barium have been shown to be influenced by temperature in some cases (Fowler et al. 1995; Hoff and Fuiman 1995; Elsdon and Gillanders 2002), but not in others (Elsdon and Gillanders 2002; Martin and Thorrold 2005; Martin and Wuenschel 2006). There is, therefore, much ambiguity concerning the relationship between temperature and elemental deposition. Moreover, it is unknown whether environmental factors, such as temperature, interact with genetic differences.

The Atlantic silverside, *Menidia menidia*, is a marine fish species with well-documented intrinsic population differences existing along a latitudinal gradient. In *M. menidia*, intrinsic differences are evidenced by countergradient variation in growth rates (Conover 1990; Conover and Present 1990). High-latitude fish have evolved a faster growth rate than low-latitude fish to compensate for the shorter growing season in the north. This genetic basis for differences in growth rates has been supported in laboratory studies (Conover 1990; Conover and Present 1990). Genetic differences in vertebral number (Billerbeck et al. 1997), growth efficiency (Present and Conover 1992), and energy allocation (Billerbeck et al. 2000) also exist along this latitudinal gradient. Such known genetic differences make *M. menidia* an excellent species to use for addressing questions concerning the influence of intrinsic factors on otolith chemistry. Specifically, we examined differences in the elemental composition (Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca) of two genetically distinct populations of *M. menidia* reared in identical conditions, and we tested for possible interactions between genotype and temperature.

Materials and methods

Genetic and temperature effects

Two genetically isolated broodstocks of *M. menidia*, one from South Carolina, USA, and another from Nova Scotia, Canada, were maintained at the Flax Pond Marine Lab, Stony Brook University, New York. The lab uses a natural seawater system that pumps water in directly from the adjacent Flax Pond (a saltwater embayment). Eggs from both stocks were reared at 21 °C. Eggs released and fertilized within the same 24 h period were collected from spawning tanks and moved to 20 L tanks until 5 days after hatch. At this time, larvae were transferred into randomly assigned experimental tanks.

Menidia menidia larvae were separated into three acid-washed 20 L flow-through plastic tanks per treatment, each individual tank containing 40 fish. These three tanks were then submerged in a larger 680 L seawater bath. Tanks were stocked with 40 fish to account for high mortality in the larval phase, but otoliths from only five fish in each tank were analyzed chemically. Fish were reared under three water temperature regimes (15, 21, and 27 °C) encompassing a range of temperatures that *M. menidia* encounter in the wild. All fish were fed *Artemia* nauplii (hatched in the same natural seawater system) and a commercial fish food (A250). Salinity was maintained at 28‰ (with a range of 27‰–29‰) for each tank throughout the duration of the experiment, and temperatures deviated very little from the targets for each treatment group. No overlap between temperatures occurred among treatment groups.

Fish were reared to approximately 30 mm standard length and then sacrificed, measured, and frozen. Ten percent of the water in the baths was exchanged weekly. Temperature and salinity were recorded daily.

Water sampling

Water samples were collected from tanks at the start, middle, and end of the experiment using a high-volume peristaltic pump (Cole Parmer) attached to an acid-washed 0.22 µm polypropylene Calyx Capsule Filter and stored in polypropylene containers. Initial samples were taken at the start of the experiment for all treatment groups at the same time, but the timing of the middle and end samples differed according to the time required for the treatment group to reach 30 mm in standard length. Samples were processed in a class 100 clean room, where they were acidified to a pH of 2 using 0.25 mL of ultrapure HCl and then refrigerated. Prior to analysis, each sample was diluted 100:1 with ultrapure HNO_3 and spiked with ^{115}In at 1 ng·g^{-1} for use as an internal standard.

Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios were analyzed in water samples using a Thermo Finnigan Element 2 inductively coupled plasma – mass spectrometer (ICP–MS). Samples were analyzed in random order. We quantified ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{86}Sr, and ^{138}Ba during each sample run. Isotope abundances were corrected using blank sample values obtained at the beginning of the run and after every sixth sample thereafter and then linearly interpolated. Values were converted to element–Ca ratios and corrected for instrument mass bias using a seawater certified reference material (High Purity Standards). Detection limits were calculated as 3 standard deviations of the blanks that were run throughout the analyses ($n = 5$). These limits were 0.01% of the average intensity for Mg, 0.31% for Ca, 2.96% for Mn, 0.48% for Sr, and 0.28% for Ba. Precision estimates (relative standard deviations), based on the seawater reference material ($n =$

5), were 2.4% for Mg, 3.3% for Ca, 2.6% for Mn, 3.2% for Sr, and 3.4% for Ba.

Otolith preparation

Five fish were randomly selected for analysis from each tank. Sagittal otoliths were extracted, cleaned of adhering tissue with Milli-Q ultrapure water, triple-rinsed in 30% H_2O_2, triple-rinsed in Milli-Q ultrapure water again, and air-dried. One randomly selected otolith from each fish was then weighed and prepared for trace element analysis. This otolith was mounted on a microscope slide and ground to the core using fine grain lapping paper. Final preparation stages were conducted on a class 100 clean bench under a laminar flow hood. Otoliths were triple-rinsed with Milli-Q water, gently scrubbed with a nylon brush, triple-rinsed with Milli-Q again, and then sonicated for 2 min. After sonication, otoliths were rinsed with Milli-Q and allowed to dry for 24 h under the laminar flow hood. Otoliths were then mounted to clean petrographic slides using double-sided tape, sealed in plastic Petri dishes, and sealed inside of double plastic bags.

Otolith analysis

We analyzed Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca from one randomly selected otolith per fish using a 213 nm Nd:YAG laser ablation system coupled to a Thermo Finnigan Element 2 ICP–MS located at the Woods Hole Oceanographic Institution. We analyzed three portions of the otolith to examine possible ontogenetic differences in elemental deposition (early, middle, and end of life). First, the laser traced out a 100 μm × 100 μm raster centered on the otolith core. Second, the laser traced another raster with identical dimensions midway between the core and the outer edge along the longest growth axis. Third, the laser traced a line 500 μm in length along the outer edge of the otolith starting at the rostrum and continuing along the ventral side of the otolith (Fig. 1). This line sampled the otolith material deposited during the final stages of the experiment. Laser spot size was 25 μm, scan speed was set at 10 μm·s^{-1}, and repetition rate was 10 Hz. We quantified ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{86}Sr, and ^{138}Ba during each sample run. Isotope abundances were corrected using blank sample values obtained at the beginning, middle, and end of a block of 10 otoliths and linearly interpolated. Values were converted to element–Ca ratios and then corrected for instrument mass bias using an otolith reference material (Yoshinaga et al. 2000) that was dissolved in 2% HNO_3 and diluted to a final Ca concentration of 40 μg·g^{-1} and also analyzed at the beginning, middle, and end of each block of 10 otoliths. Detection limits were calculated as 3 standard deviations of the blanks that were run throughout the analyses (n = 38). These limits were 0.1% of the average intensity for Mg, 0.1% for Ca, 15% for Mn, 0.6% for Sr, and 0.7% for Ba. External precision (relative standard deviation) was calculated by running a second otolith reference standard (Sturgeon et al. 2005) as an unknown and was 1.6% for Mg:Ca, 3.9% for Mn:Ca, 1.7% for Sr:Ca, and 4.1% for Ba:Ca.

Partition coefficients (D_{Me}) describe otolith chemical concentrations relative to water chemical concentrations. We calculated D_{Me} by dividing the metal–calcium (Me:Ca) ratio measured in an otolith by the Me:Ca ratio measured in the treatment tank water. Me:Ca values from the five individual fish were averaged within tanks, and then the three tank averages were used as replicates for each of the treatments.

Growth rates

Mean growth rates per treatment were calculated by dividing the fish's standard length by the number of days from birth to sacrifice. All fish were spawned within the same 24 h period, hatched within 1–2 days of each other, and randomly assigned to treatment tanks, so differences among treatments in fish size at the start of the experiment (age 5 days) are expected to be minimal. Hence, size at the end of the experiment is a direct measure of individual growth rate.

Statistical analysis

A repeated measures analysis of variance (ANOVA) was used to test for significant ($p < 0.05$) differences in Me:Ca$_{[water]}$, Me:Ca$_{[otol]}$, and D_{Me} among temperature and population treatments. Temperature and population were treated as categorical variables, with the position of the otolith measurement or date of the water sample (beginning, middle, and end of experiment) as the repeated measures component. Me:Ca$_{[water]}$, Me:Ca$_{[otol]}$, and D_{Me} were the dependent variables in the analyses. Values were log-transformed to meet assumptions of normality.

Because differences in precipitation rate have the potential to confound factors influencing deposition of biogenic aragonite (Stecher et al. 1996; Martin and Thorrold 2005), we used otolith mass as a proxy for otolith precipitation rate. Correlation analysis was used to test if mean Me:Ca$_{[otol]}$ and D_{Me} values per tank were related to otolith precipitation or fish somatic growth rates.

Results

Survivorship was high in all treatment groups except for the South Carolina population reared at 15 °C. In this treatment, one replicate tank had 100% mortality, allowing for no samples to be collected from this group. In another replicate of this treatment group, otoliths could not be detected (see section on Otolith mass and somatic growth rate below for a further description). Therefore, analysis of the South Carolina population at 15 °C is based on five fish from one tank. Differences in mean size, otolith mass, and age at sacrifice among treatment groups are presented (Table 1).

Water chemistry

Elemental concentrations (Me:Ca$_{[water]}$) in the tank water varied temporally within tanks for each of the ratios, but did not differ significantly among temperature or population treatment groups (Fig. 2). We detected no significant differences for any Me:Ca$_{[water]}$ ratio. It should be noted that the greater variation observed towards the end of the experiment could be the result of differences in the time of sampling. As noted in Table 1, treatment groups differed in the time required for fish to reach an average of 30 mm standard length, therefore sample dates for the middle and end of the experiment differed among groups.

Fig. 1. Sampling areas on the otoliths. Three regions were traced with the laser: (*a*) 100 μm × 100 μm raster centered on the otolith core, (*b*) 100 μm × 100 μm raster midway between the core and the outer edge along the longest growth axis, and (*c*) 500 μm long line along the outer edge of the otolith starting at the rostrum and continuing along the ventral side of the otolith. Note: image is not to scale.

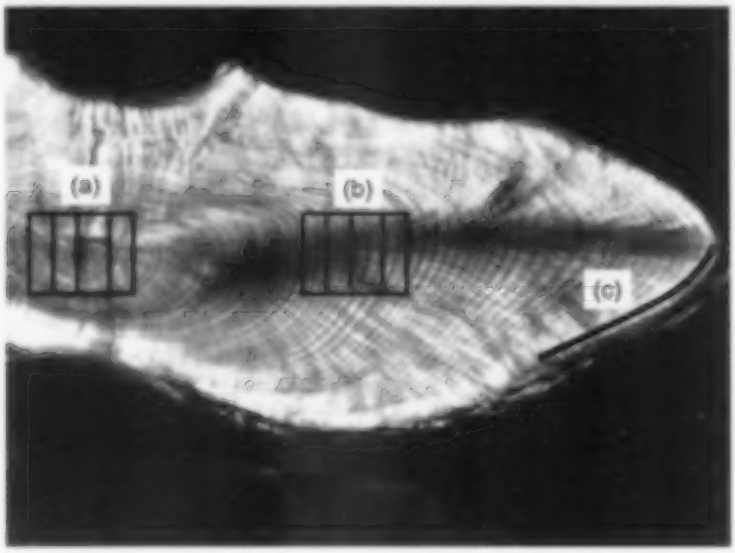


Table 1. Differences in mean standard length, otolith mass, and age at sacrifice among treatment groups.

Population	Temperature (°C)	Mean standard length (mm)	Mean otolith mass (mg)	Age at sacrifice (days)
NS	15	28.6	0.032	89
	21	28.2	0.043	47
	27	29.5	0.095	44
SC	15	27.8	0.036	166
	21	27.7	0.046	74
	27	29.9	0.078	58

Note: NS, Nova Scotia; SC, South Carolina.

Mg:Ca and D_{Mg}

Otolith Mg:Ca values differed significantly between Nova Scotia and South Carolina populations (Table 2, Fig. 3), with higher values in each temperature treatment for Nova Scotia fish. We detected no significant temperature effect on Mg:Ca. Mg:Ca ratios differed significantly according to the position on the otolith, but neither interaction effect with population nor with temperature was significant (Table 2).

The Mg partition coefficient (D_{Mg}) also differed significantly between populations, with Nova Scotia again showing higher values for each temperature treatment (Table 3, Fig. 4). We detected no significant relationship with temperature but D_{Mg} varied significantly with otolith position (Table 3, Fig. 4). A significant interaction between position and population precluded interpretation of these effects in isolation.

Mn:Ca and D_{Mn}

Both Mn:Ca ratios and D_{Mn} varied significantly with population, but not with temperature (Tables 2 and 3, Figs. 3 and 4). Again, Nova Scotia showed higher values for each temperature treatment. D_{Mn} also increased with increasing temperature. Mn:Ca ratios and D_{Mn} differed significantly according to otolith position, but no significant position and population interaction was detected (Fig. 5).

Sr:Ca and D_{Sr}

Sr:Ca ratios did not differ significantly between populations, although Nova Scotia again showed higher values (Table 2, Fig. 3). We did detect a significant relationship with temperature. Both South Carolina and Nova Scotia Sr:Ca values increased with increasing temperature, and the difference was most pronounced at 27 °C. Furthermore, a significant temperature × otolith position interaction was present, which precluded interpretation of these effects in isolation (Fig. 5).

D_{Sr} values did not differ significantly between populations, but a significant relationship with temperature was detected in addition to a significant temperature × position interaction (Table 3, Fig. 4). The difference between temperatures was only pronounced at 27 °C, with D_{Sr} increasing for both populations. As with the Sr:Ca values, a significant temperature × otolith position interaction was present.

Fig. 2. $Me:Ca_{[water]}$ ratios measured in experimental tank water throughout the duration of the experiment. Mean treatment ratios (± standard error, SE) are shown for the tanks of Nova Scotia (solid symbols) and South Carolina (open symbols) populations reared at 15 °C (circles), 21 °C (squares), and 27 °C (triangles).

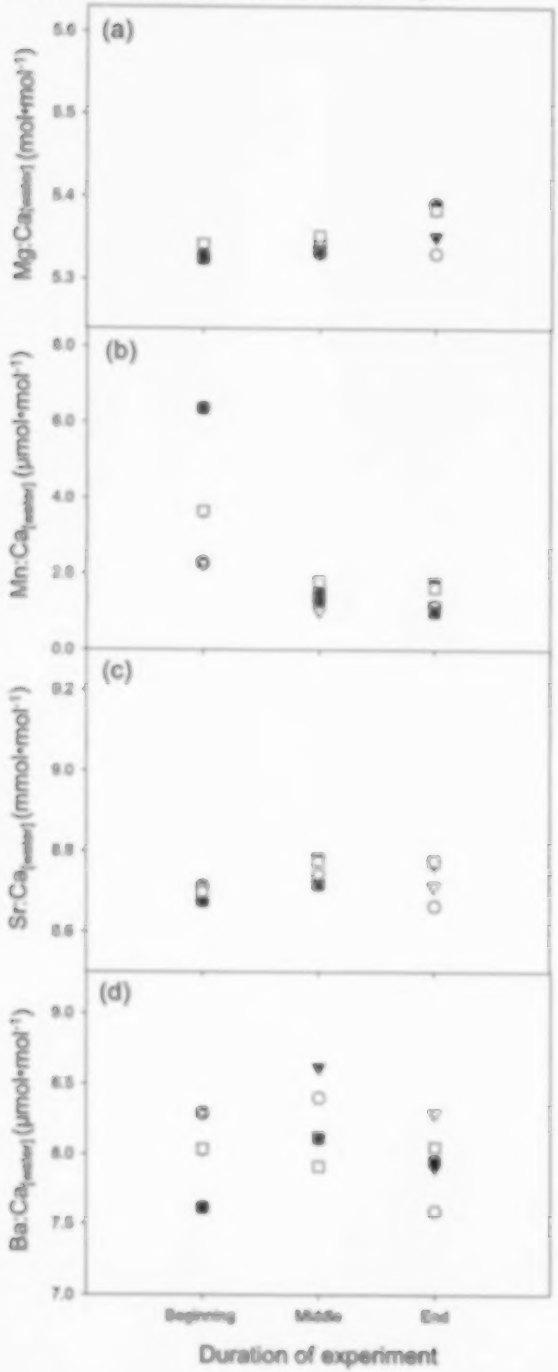


Table 2. Results of repeated measures analysis of variance (ANOVA) testing effects of population (Pop) and temperature (Temp) on $Me:Ca_{[otol]}$.

	df	MS	*F*	*p*
Mg:Ca				
Pop	**1**	**3.324**	**12.90**	**0.005**
Temp	2	0.342	1.33	0.308
Pop×Temp	2	0.619	2.40	0.141
POS	**2**	**0.881**	**15.35**	**<0.001**
POS×Pop	2	0.803	1.40	0.270
POS×Temp	4	0.088	1.53	0.233
POS×Pop×Temp	4	0.070	1.21	0.336
Mn:Ca				
Pop	**1**	**0.508**	**7.22**	**0.022**
Temp	2	0.104	1.48	0.273
Pop×Temp	2	0.010	0.14	0.867
POS	**2**	**0.408**	**7.79**	**0.003**
POS×Pop	2	0.095	1.81	0.189
POS×Temp	**4**	**0.197**	**3.75**	**0.020**
POS×Pop×Temp	4	0.038	0.72	0.589
Sr:Ca				
Pop	1	0.003	0.81	0.389
Temp	**2**	**0.040**	**10.50**	**0.003**
Pop×Temp	2	0.000	0.10	0.906
POS	**2**	**0.007**	**4.53**	**0.024**
POS×Pop	2	0.005	3.44	0.052
POS×Temp	**4**	**0.005**	**3.78**	**0.019**
POS×Pop×Temp	4	0.002	1.56	0.222
Ba:Ca				
Pop	**1**	**0.234**	**13.22**	**0.005**
Temp	2	0.071	4.04	0.052
Pop×Temp	2	0.019	1.08	0.375
POS	**2**	**0.316**	**20.85**	**<0.001**
POS×Pop	2	0.020	1.35	0.282
POS×Temp	**4**	**0.069**	**4.58**	**0.009**
POS×Pop×Temp	4	0.032	2.11	0.118

Note: Otolith position (POS) is treated as the repeated measure variable. Me:Ca data were log-transformed. Significant ($p < 0.05$) relationships are highlighted in bold; df, degrees of freedom; MS, mean square.

Ba:Ca and D_{Ba}

Ba:Ca ratios were significantly different among populations but not for temperatures. Fish from Nova Scotia showed higher Ba:Ca ratios than fish from South Carolina. Significant differences also existed within an otolith based on location (Figs. 3 and 5, Table 2).

D_{Ba} values were significantly related to population and temperature, but there was no significant population × temperature interaction (Fig. 4, Table 3). Nova Scotia showed higher D_{Ba} values for each temperature. The pattern was similar to that observed for D_{Sr}, and the greatest difference between populations and temperature was observed at 27 °C. The relationship between D_{Ba} and temperature appeared to be curvilinear, with the fished reared at 15 and 27 °C showing the highest values. A significant interaction term was detected for temperature and otolith position, making it difficult to interpret these effects individually.

Fig. 3. Mean (± standard error, SE) Me:Ca ratios measured in the otoliths of *Menidia menidia* populations from Nova Scotia (solid symbols) and South Carolina (open symbols) reared at different temperatures. Otolith measurements represent an average of all three positions.

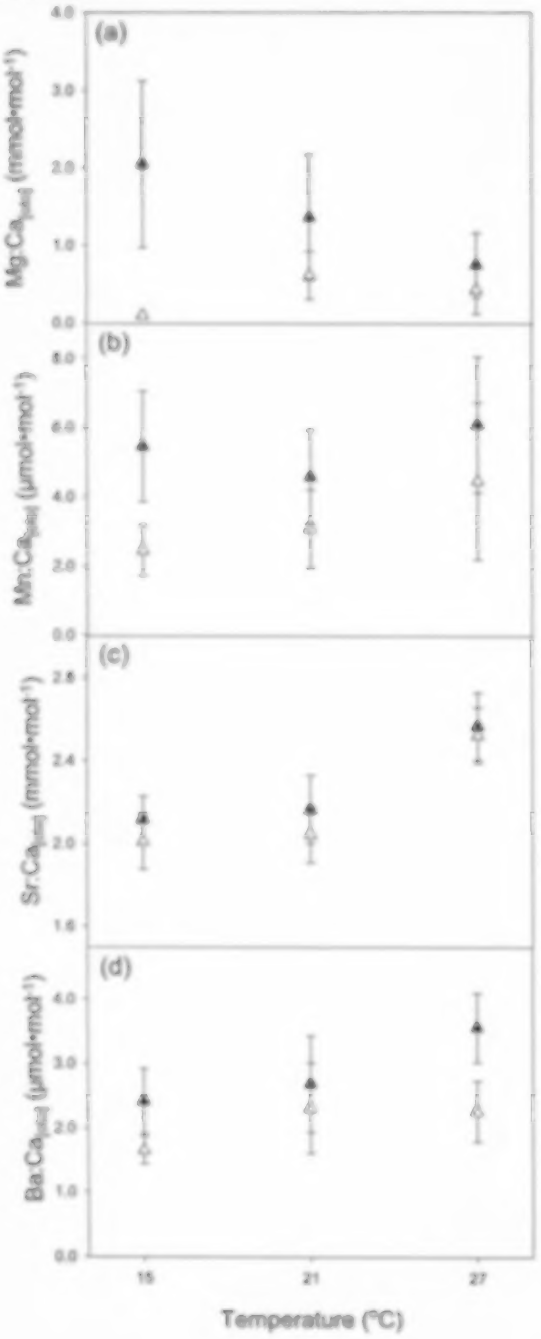


Table 3. Results of repeated measures analysis of variance (ANOVA) testing effects of population (Pop) and temperature (Temp) on D_{Me}.

	df	MS	*F*	*p*
D_{Mg}				
Pop	**1**	**0.000**	**6.47**	**0.029**
Temp	2	0.000	0.64	0.548
Pop×Temp	2	0.000	1.26	0.326
POS	**2**	**0.000**	**9.91**	**0.001**
POS×Pop	**2**	**0.000**	**3.74**	**0.041**
POS×Temp	4	0.000	0.67	0.621
POS×Pop×Temp	4	0.000	1.27	0.314
D_{Mn}				
Pop	**1**	**17.078**	**4.81**	**0.053**
Temp	2	5.611	1.58	0.253
Pop×Temp	2	0.284	0.08	0.924
POS	**2**	**8.896**	**3.59**	**0.047**
POS×Pop	2	4.659	1.86	0.182
POS×Temp	4	5.423	2.17	0.110
POS×Pop×Temp	4	0.633	0.25	0.905
D_{Sr}				
Pop	1	0.001	1.03	0.334
Temp	**2**	**0.014**	**9.72**	**0.005**
Pop×Temp	2	0.000	0.10	0.903
POS	**2**	**0.003**	**8.08**	**0.003**
POS×Pop	**2**	**0.002**	**5.18**	**0.015**
POS×Temp	**4**	**0.001**	**3.77**	**0.019**
POS×Pop×Temp	4	0.001	2.49	0.078
D_{Ba}				
Pop	**1**	**0.106**	**11.52**	**0.007**
Temp	**2**	**0.098**	**10.58**	**0.003**
Pop×Temp	2	0.014	1.55	0.259
POS	**2**	**0.113**	**9.920**	**0.001**
POS×Pop	2	0.018	1.43	0.261
POS×Temp	**4**	**0.120**	**9.71**	**<0.001**
POS×Pop×Temp	4	0.006	0.52	0.722

Note: Otolith position (POS) is treated as the repeated measure variable. Significant ($p < 0.05$) relationships are highlighted in bold; *df*, degrees of freedom; MS, mean square.

Otolith mass and somatic growth rate

Fish were sacrificed when individual treatment groups reached an average of 30 mm in total length. Growth rates were significantly different among temperature and population treatment groups (Tables 4 and 5). Fish from the Nova Scotia population grew faster than fish from the South Carolina population at all temperatures.

We used otolith mass as a proxy for precipitation rate. Correlation analysis revealed no significant relationships between otolith masses and Mg:Ca (r = –0.306, p = 0.249), Mn:Ca (r = –0.023, p = 0.933), Sr:Ca (r = –0.318, p = 0.230), and Ba:Ca (r = –0.377, p = 0.150) ratios. Correlation analysis also revealed no significant relationships between otolith mass and D_{Mg} (r = 0.168, p = 0.533), D_{Mn} (r = 0.199, p = 0.461), D_{Sr} (r = 0.339, p = 0.199), and D_{Ba} (r = 385, p = 0.140).

Somatic growth rate initially appeared to influence D_{Mg}, but after the Bonferroni correction for multiple correlations

Fig. 4. Mean (± standard error, SE) partition coefficients (D_{Me}) measured in the otoliths of *Menidia menidia* populations from Nova Scotia (solid symbols) and South Carolina (open symbols) reared at different temperatures. Otolith measurements represent an average of all three positions.

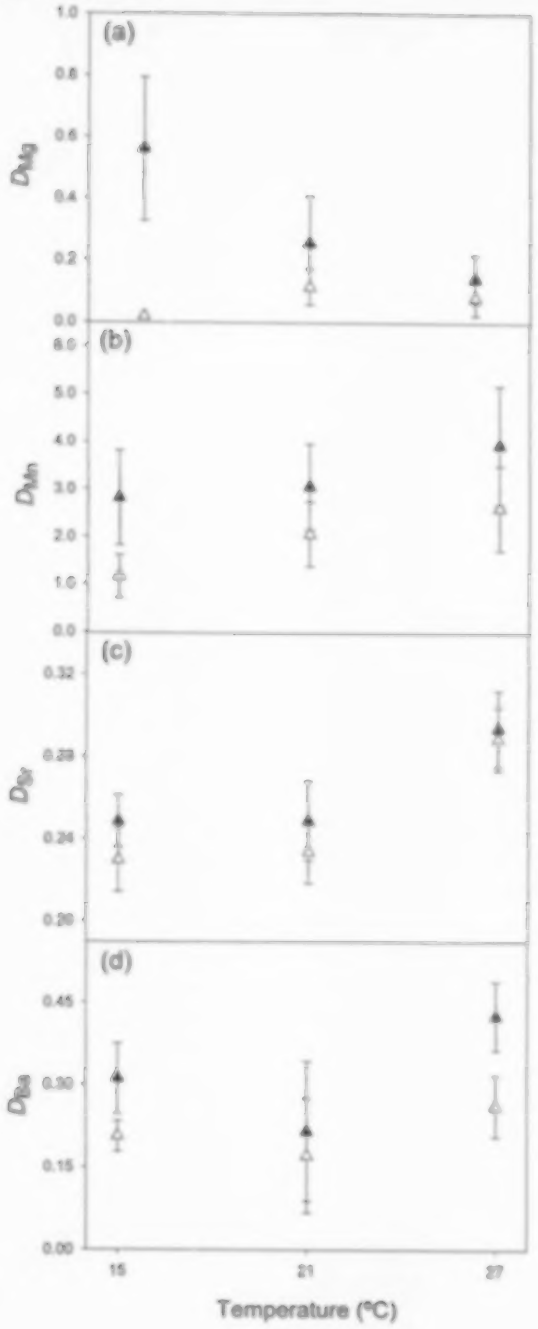
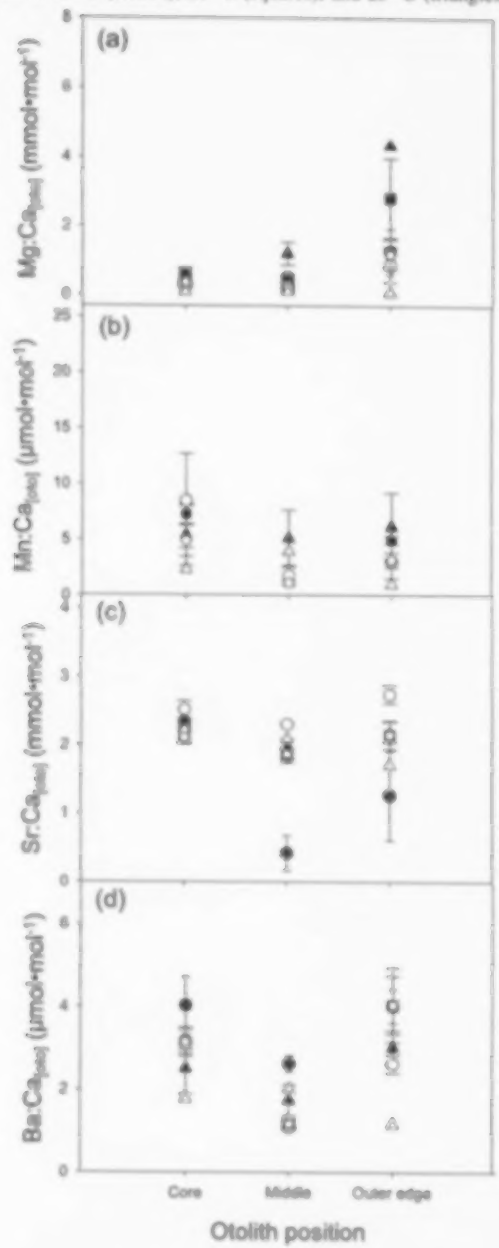


Fig. 5. Mean (± standard error, SE) Me:Ca ratios measured in different locations of *Menidia menidia* otoliths from Nova Scotia (solid symbols) and South Carolina (open symbols) populations reared at 15 °C (circles), 21 °C (squares), and 25 °C (triangles).



was applied, this relationship was no longer significant (r = 0.527, p = 0.036, Bonferroni adjusted α = 0.0125). No obvious relationship between somatic growth rate and the other partition coefficients was evident (D_{Mn}: r = 0.346, p = 0.189; D_{Sr}: r = 0.130, p = 0.630; D_{Ba}: r = 0.241, p = 0.369). Similarly, somatic growth rate did not significantly influence Me:Ca ratios. Initial significance tests with D_{Sr}

Table 4. Average growth rates for *M. menidia* from different populations reared under different temperature (Temp) regimes.

Population	Temp (°C)	Mean growth (mm·day^{-1})	SE
NS	15	0.30	0.005
	21	0.53	0.027
	27	0.62	0.007
SC	15	0.19	—
	21	0.35	0.007
	27	0.47	0.017

Note: NS, Nova Scotia; SC, South Carolina.

Table 5. Results of a two-way analysis of variance (ANOVA) testing the effects of population (Pop) and temperature (Temp) on growth rate.

	df	MS	F	p
Pop	1	0.069	308.16	**<0.001**
Temp	2	0.089	398.93	**<0.001**
Pop×Temp	2	0.001	4.85	**0.033**

Note: Significant ($p < 0.05$) relationships are highlighted in bold; df, degrees of freedom; MS, mean square.

and D_{Ba} were no longer significant after the Bonferroni correction was applied (D_{Sr}: $r = -0.57$, $p = 0.021$, Bonferroni adjusted $\alpha = 0.0125$; D_{Ba}: $r = -0.553$, $p = 0.026$, Bonferroni adjusted $\alpha = 0.0125$). D_{Mg} ($r = 0.234$, $p = 0.384$) and D_{Mn} ($r = -0.192$, $p = 0.476$) were not significantly influenced by somatic growth rate.

While otolith mass and Me:Ca and D_{Me} were not significantly related, a significant relationship was observed between otolith mass and growth rate ($r = 0.759$, $p = 0.001$). The faster growing fish had visibly larger otoliths than the slower growing fish of similar sizes. For example, the average otolith mass for the fastest growing fish (96 µg; the Nova Scotia population reared at 27 °C) was almost triple that of the slowest growing fish (36 µg; the South Carolina population reared at 15 °C). In fact, otoliths could not be located in any of the fish reared in tank no. 3 from the South Carolina fish reared at 15 °C, so only one tank was used for analysis in this treatment group.

Discussion

Considerable effort has gone into understanding factors that influence the chemical composition of otoliths. While previous focus has largely been on environmental (e.g., ambient water chemistry, temperature, salinity) and physiological (e.g., growth and maturation) factors, this is the first study to demonstrate that differences in otolith chemistry among fish populations have a genetic component. Genetically distinct populations of *M. menidia* raised under identical laboratory conditions had significant differences in Me:Ca ratios and Mg, Mn, and Ba partition coefficients. Given that salinity, temperature, water chemistry, and diet were all constant among treatments, some other factors intrinsic to each population must have been responsible for the observed differences. We found significant differences in somatic growth rates between populations (previously documented in Conover and Present 1990), but we found no significant correlations between somatic growth and elemental concentrations or partition coefficients. Therefore, while growth cannot be ruled out as a contributing factor, it is clearly not the sole cause of the observed population differences.

We found that Mg, Sr, and Ba partition coefficients were less than 1 in all treatments, demonstrating at least some level of discrimination of each of the elements compared with Ca. The exact mechanism and location of this discrimination is not known. Ions are derived from seawater, and they must cross intestinal or branchial membranes to get into blood plasma and then pass through the endolymphatic membrane to arrive at the site of aragonite deposition on the otolith surface. There is certainly the potential for elemental discrimination if dissolved ions travel through Ca channels across any or all of these membranes. Whatever the mechanism, it is clear that Mg:Ca, Sr:Ca, and Ba:Ca values in otoliths are influenced by physiological processes as well as the physiochemical properties of the ambient environment (Martin and Thorrold 2005). Moreover, these physiological processes appear to be under some degree of genetic control for Mg:Ca and Ba:Ca, but not for Sr:Ca. The lack of a detectable genetic component for Sr deposition suggests that Sr:Ca ratios in *M. menidia* otoliths likely record environmental histories (e.g., Bath et al. 2000), albeit in a complex manner.

Unlike the other three elements we assayed, Mn partition coefficients were always greater than 1, indicating that Mn is being concentrated in *M. menidia* otoliths. The mechanisms regulating Mn deposition in otoliths are not clear. Although ambient Mn concentrations may play some role, it has not always been possible to detect significant correlations between $Mn_{[otol]}$ and $Mn_{[water]}$ (Elsdon and Gillanders 2003). Dorval et al. (2007) reported a nonlinear relationship between $Mn_{[otol]}$ and $Mn_{[water]}$ in spotted seatrout (*Cynoscion nebulosus*) and D_{Mn} values (14.08) that were even higher than the ones we detected in *M. menidia*. Given the physiological complexities regulating otolith deposition, it is perhaps not surprising that the underlying mechanisms behind biological regulation may also be subject to intrinsic differences among populations.

We found significant temperature effects on partition coefficients of both Sr and Ba. Values were highest at 27 °C, but the temperature relationships for both elements were nonlinear. Numerous studies have documented environmental influences on Sr:Ca and Ba:Ca ratios in otoliths (e.g., Elsdon and Gillanders 2004; Walther and Thorrold 2006; but also see Kalish 1989). However, most researchers have reported that temperature did not significantly influence D_{Ba} in fish otoliths (Bath et al. 2000; Martin and Thorrold 2005; but see Elsdon and Gillanders 2004). Temperature does, however, exert some influence on Ba:Ca ratios in inorganic aragonite (Gaetani and Cohen 2006), and temperature effects on barium have been documented in aragonite from mollusk species (Leng and Pearce 1999; Zacherl et al. 2003) and the common cuttlefish (*Sepia officinalis*) (Zumholz et al. 2007). Clearly more work is needed before generalizations can be drawn on temperature effects on otolith Ba:Ca ratios.

Ontogenetic effects also appeared to significantly influence the chemical composition of *M. menidia* otoliths. The presence of age (or ontogenetic) differences have been previously reported in a variety of fish species (Kalish 1989; Sadovy and Severin 1992; Fowler et al. 1995). Other studies have reported clear peaks in elemental ratios associated with distinct life history transitions, including eel metamorphosis (Tzeng et al. 1997) and larval settlement (Hamilton and Warner 2009). Hamilton and Warner (2009) reported that Ba:Ca ratios were six times higher after settlement of bluehead, *Thalassoma bifasciatum*, on coral reefs. This sharp increase was assumed to be due to environmental changes, but the role of ontogenetic effects has not been fully tested.

In *M. menidia*, transformation from the larval to juvenile life stage occurs before 20 mm total length (Wang 1974), so the three portions of the otolith analyzed in this study correspond to the core, larval, and juvenile stages. Although the elemental patterns observed in *M. menidia* differed among treatment groups, many of the overall patterns in relation to age were similar to those reported in other studies (Fowler et al. 1995; Hamilton and Warner 2009). Generally, Me:Ca values were highest in the core area, decreased towards the middle of the otolith, and increased again on the outer edge. Ruttenberg et al. (2005) detected elevated elemental ration (especially for Mn) in the core of the otolith that appeared to be related to maternal effects. They speculated that these effects combined with other findings of lower Ca concentrations in the center of the otolith (Dove et al. 1996), and increased levels of proteins needed for initial otolith calcification (Murayama et al. 2004) may explain higher metal concentrations in otolith cores. These results may also explain some of the elevated levels in the core region of *M. menidia* otoliths as well as the high D_{Mn} values we found. While the increase in the core region was not as sharp as those reported in Ruttenberg et al. (2005), we ablated a 100 μm × 100 μm raster around the core region, and any sharp increase in the core was likely dampened. Therefore, although we detected significant position effects for each Me:Ca ratio analyzed, the lack of significant population and position interactions for all Me:Ca ratios, as well as D_{Mn} and D_{Ba} values, implied that the population differences in otolith chemistry that we observed were not simply the result of age differences.

The presence of intrinsic differences in otolith elemental deposition has important implications for the interpretation and utility of otolith data. First, it may shed some light on conflicting results in the literature. Second, knowledge of genetic effects may help our ability to reconstruct environmental and physiological histories as well as increase our understanding of the otolith deposition process. Third and most importantly, these findings suggest that genetic differences among populations in otolith chemistry may be detectable even in the absence of environmental differences in habitat. If so, the utility of otolith chemistry to discern the population structure of fishes is even more powerful than currently appreciated.

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Intracohort and intercohort spatial density dependence in juvenile brown trout (*Salmo trutta*)

Eli Kvingedal and Sigurd Einum

Abstract: An increased mobility with age can be expected for many organisms, which will reduce the potential for spatial density dependence. Here we quantify the extent of spatial density dependence for two juvenile age classes of brown trout (*Salmo trutta*) that differ in their dispersal abilities. As predicted, spatial variation in intracohort density had a strong effect on the performance of underyearlings, but not on yearlings. However, rather surprisingly, local underyearling density influenced the energetics of the older age class to the same extent as their own. Thus, yearlings do not appear to prioritize growth rate per se as a cue in movement decisions. The spatial patterns of densities and performance are consistent with older fish distributing themselves primarily according to preferred abiotic variables that also influence energetics. Such decisions would remove any relation between local density of their own age class and growth performance. However, because of competitive effects from the patchily distributed younger age class, spatial homogenization of competition intensities appears to be constrained. Variation in yearling body mass also increased with density of their own age class, indicating that even though the average effect of intracohort competition was absent, the individual response was asymmetric.

Résumé : On peut s'attendre à une mobilité accrue en fonction de l'âge chez de nombreux organismes, ce qui réduit leur dépendance spatiale potentielle reliée à la densité. Nous mesurons ici l'importance de la densité-dépendance spatiale chez deux classes juvéniles de truites brunes (*Salmo trutta*) qui diffèrent par leur capacité de dispersion. Comme prédit, la variation de la densité au sein des cohortes a un effet important sur la performance des individus âgés de moins d'un an, mais pas sur celle des individus d'un an. Cependant de manière inattendue, la densité locale des individus de moins d'un an influence le bilan énergétique de la classe plus âgée au même titre que leur densité propre. Ainsi, les individus d'un an ne semblent pas donner une priorité au taux de croissance per se comme signal dans leurs décisions de déplacement. Les patrons spatiaux de densité et de performance sont compatibles avec la répartition des poissons plus âgés en fonction principalement de leurs variables abiotiques préférées qui influencent aussi leur bilan énergétique. De telles décisions élimineraient toute relation entre la densité locale de leur propre classe d'âge et leur performance de croissance. Cependant, à cause des effets compétitifs de la répartition contagieuse de la classe d'âge plus jeune, l'homogénéisation des intensités de compétition semble réduite. La variation de la masse corporelle des individus d'un an augmente aussi en fonction de la densité de leur propre classe d'âge, ce qui indique que, même s'il n'y a pas d'effet moyen de la compétition à l'intérieur de la cohorte, la réaction individuelle est asymétrique.

[Traduit par la Rédaction]

Introduction

Under an ideal free distribution (reviewed by Tregenza 1995), efficient utilization of resources available within the spatial range of the population is ensured. However, for many organisms, such distributions are unlikely, in which case some areas are likely to be underutilized (or not utilized at all), whereas other organisms experience crowding and associated negative effects on fitness (e.g., Abrahams 1986; Polivka 2005). One consequence of this can be that the population does not reach its potential carrying capacity (i.e., the number of adults that can be produced).

The issue of deviations from ideal distributions is of particular interest for age-structured populations. These may show changes in the age-specific carrying capacity due to ontogenetic niche shifts (Elliott 1990; Armstrong and Nislow 2006) and (or) changes in per capita resource requirements. For example, ontogenetic increases in per capita resource requirements are the driving force behind the self-thinning pattern observed in plants (reviewed by Reynolds and Ford 2005) and sessile (reviewed by Guiñez 2005) and mobile animals (Begon et al. 1986; Latto 1994). A less recognized factor that can cause similar ontogenetic changes in carrying capacity is an age-specific ability to homogenize fitness over space. In particular, older and more mobile individuals may be better able to distribute themselves accord-

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E. Kvingedal.[1] Norwegian Institute for Nature Research, Tungasletta 2, NO-7485 Trondheim, Norway.
S. Einum. Centre for Conservation Biology, Department of Biology, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway.

[1]Corresponding author (e-mail: eli.kvingedal@nina.no).

ing to the spatial distribution of resource levels (Armstrong 2005), thereby reducing any spatial density dependence (Einum et al. 2008*b*). This mechanism would cause differences in the potential for different age classes to efficiently utilize the total extent of resources available within the population range and hence influence age-specific carrying capacities.

Stream-dwelling salmonids are well suited for studying the potential role of age-specific spatial distributions and the importance that these may have for age-specific carrying capacities. These species deposit eggs in gravel nests located in specific spawning grounds with appropriate habitat characteristics (Fleming 1998). As the salmonid fry emerge from the gravel, they settle in suitable microhabitats close to the nest and start to defend feeding territories (Elliott 1990). Spatial variation in initial densities during emergence primarily influences mortality rates, with little effects on growth or dispersal of those that survive (Einum et al. 2006). As the summer progresses, spatial variation in density becomes more likely to influence growth rates (Einum et al. 2006), as found in many natural populations (Jenkins et al. 1999; Grant and Imre 2005). Local competition may also to a larger extent cause increased dispersal away from areas of high density, suggesting an ontogenetic increase in dispersal abilities (Einum et al. 2006). For yearling and overyearling salmonids, high density in closed systems (i.e., tanks or experimental stream channels) causes reduced growth rates, indicating the potential for spatial density dependence for older fish (Jenkins et al. 1999). However, because of the ontogenetic increase in dispersal abilities that is observed even over short periods (months) of their first year, one may predict that older juveniles in natural systems are able to spatially homogenize competitive intensities and thereby be better able to utilize their resources across space. Tests of this prediction do, however, require larger scale field studies of natural populations that allow for fish movements.

Here we use spatially structured data from a natural population of brown trout (*Salmo trutta*) to test whether yearlings (i.e., 1 year old) and underyearlings differ in the degree to which dispersal contributes to homogenize experienced habitat quality. We tested for effects of local population density on body size (body length, wet mass, and dry mass) as well as a proxy for energy density (ratio of dry to wet mass, Hartman and Brandt 1995). The latter measure is a more dynamic measure of condition that better reflects recent food availability (Weber et al. 2003). Hence, we circumvent the potential problems associated with using measures of body mass, which will in part be determined by conditions experienced the previous growth season. To allow for analyses of intercohort and interspecific competition, densities were subdivided into age- and species-specific components (underyearlings and yearlings of brown trout and Atlantic salmon (*Salmo salar*), respectively). Based on the studies suggesting ontogenetic increases in mobility mentioned above, we predict that yearlings should show a lower degree of spatial density dependence with respect to body mass and energy density than underyearlings. To obtain information on individual differences in the response to density, we also tested for effects of spatial density on the within-site variation in performance.

Fig. 1. Sampling localities in the River Gråelva, Norway.

Materials and methods

Study area and data collection

Data were collected in the River Gråelva (63°29′N, 11°05′E), a tributary of the River Stjørdalselva in central Norway (Fig. 1). Anadromous brown trout dominate this river, while Atlantic salmon juveniles are found mainly in the lower parts of the study area and with only scattered distribution in the upper regions (Berger et al. 1997).

Three-pass electrofishing was conducted in 17 survey stations (Fig. 1) during 25–29 June 2005. Each station was 20 m long and on average 7 m wide (Table 1). Up to 20 randomly sampled individual juveniles from each age class (divided into underyearlings and yearlings) of both trout and salmon were killed at each station by a overdose of anaesthetic (clove oil) and measured for body mass (wet mass, ±0.01 g) and total length (±1 mm). The fish were kept on ice for 3–12 h until transferred to storage at –20 °C. The age of sampled fish was determined by scale analysis. Dry body mass was measured for each individual after drying at 70 °C for a minimum of 48 h to ensure that a stable mass had been achieved.

Data analysis

Densities of trout and salmon underyearlings and yearlings (with the latter including a small number of overyearlings) were calculated for each station by Zippin's removal method (Bohlin et al. 1989). Age-specific capture efficiencies were estimated for trout and salmon separately when total catch was more than 25 individuals. If fewer than 25 individuals were caught within a species and age class, the numbers of trout and salmon within the same age class (underyearlings or yearlings) were pooled during estimation of capture efficiency. For stations where estimates did not converge or the total capture within the age class was lower than 25, the average river capture efficiency for that age class was used.

To test for density effects on trout performance, we used stepwise simplification of linear mixed effects models (LMMs) according to Zuur et al. (2009). Mixed effect models allow for analysis of individual data when measurements

Table 1. Sampling area (m^2), captured number of brown trout and Atlantic salmon (N), estimated capture rate (p), densities (D (100 m^{-2}) ± 1.96 standard error, SE), and average body mass (M (g) ± standard deviation, SD) for the survey stations (St.) in the River Gråelva.

		Brown trout								Atlantic salmon							
		Underyearlings				Yearlings				Underyearlings				Yearlings			
St.	Area	N	p	D	M	N	p	D	M	N	p	D	M	N	p	D	M
1	234	101	0.76	43.8±12	2.16±0.40	16	0.76	6.9	15.4±5.76	197	0.66	87.6±3.4	1.29±0.24	10	0.76	4.3	11.0±5.53
2	112	52	0.53	51.8±8.2	2.49±0.46	36	0.80	32.4±1.1	15.6±6.76	9	0.53	9	1.64±0.34	25	0.55	24.6±5.1	12.3±5.59
3	117	10	0.55	9.5	2.62±0.36	24	0.67	21.3±2.2	17.4±6.49	0	—	—	—	10	0.62	9.1	11.4±4.02
4	94	75	0.66	83.3±5.1	2.54±0.66	8	0.67	8.9	15.6±3.75	0	—	—	—	2	0.67	2.2	20.1±2.05
5	127	21	0.55	18.3	2.66±0.59	23	0.66	18.9	14.9±4.98	0	—	—	—	25	0.58	21.3±3.6	12.5±4.40
6	210	63	0.66	31.2±2.1	2.72±0.45	47	0.69	23.1±1.6	14.8±5.33	0	—	—	—	1	0.66	0.5	10.3
7	185	140	0.51	85.5±9.0	2.32±0.55	37	0.58	21.6±3.1	13.8±4.38	0	—	—	—	9	0.66	5.1	17.3±3.24
8	266	103	0.61	41.1±2.9	2.66±0.54	71	0.63	28.1±2.1	15.5±5.38	0	—	—	—	7	0.64	2.8	14.9±5.11
9	250	148	0.68	61.2±2.5	2.69±0.68	59	0.69	24.3±1.4	18.3±7.33	3	0.68	1.2	1.79±0.32	10	0.67	4.1	17.5±5.55
10	245	366	0.56	163.5±8.3	2.03±0.55	14	0.67	5.9	9.87±2.81	3	0.56	1.3	1.12±0.24	9	0.67	3.8	12.8±3.50
11	61	41	0.28	106.8	1.53±0.49	21	0.62	36.8	12.8±4.45	0	—	—	—	31	0.61	54.5±7.2	7.07±2.32
12	101	208	0.36	278.9±55.3	1.64±0.62	22	0.82	21.9	13.6±4.91	1	0.36	1.3	0.49	12	0.82	12	10.9±3.13
13	77	145	0.38	247.6±51.8	1.57±0.77	22	0.69	29.6	9.46±3.92	0	—	—	—	9	0.69	12.1	10.8±1.24
14	73	253	0.49	399.2±35.5	1.80±0.75	30	0.77	41.6±2.0	13.5±6.46	0	—	—	—	8	0.75	11.1	10.2±3.02
15	64	201	0.42	392.3±56.6	1.58±0.56	43	0.68	70.1±5.2	13.6±5.17	0	—	—	—	6	0.72	9.7	11.5±4.14
16	118	46	0.79	39.5±1.3	2.48±0.47	23	0.67	20.3	16.1±4.69	0	—	—	—	1	0.67	0.9	15.6
17	99	11	0.55	12.3	2.34±0.43	6	0.67	6.3	13.7±2.85	1	0.55	1.1	1.49	0	—	—	—

are spatially or temporally correlated (Crawley 2007; Zuur et al. 2009). Including sampling station as a random factor in our LMMs induces a correlation structure between observations within the same station. To complement these analyses, we also performed analyses of covariance (ANCOVAs) on average values of performance. All statistical modelling was performed in the statistical software package R 2.10.0 for Windows (http://www.r-project.org/). The mixed models were analysed by the "lme" function of the "nlme" package (Pinheiro et al. 2009).

We started by testing the influence of age and fish densities (i.e., underyearling and yearling trout and salmon densities). The starting model included two-way interactions between age and age- and species-specific densities, allowing for the possibility that underyearlings and yearlings respond differently to competition from individuals of a certain age and species. Hence, the fixed effect structure of the starting LMM was given by the following: body mass ~ age + TUY + TY + SUY + SY + age × TUY + age × TY + age × SUY + age × SY, where TUY and TY were trout underyearling and yearling densities, respectively, and SUY and SY were corresponding estimates for salmon. Previous studies have reported a negative power relationship between population density and growth (Jenkins et al. 1999); hence, we log transformed both body mass and age- and species-specific densities prior to the analysis. We used a $\log(x + 1)$ transformation because of zeros in the data.

Initial exploration of the model residuals revealed variance heterogeneity, which was controlled for by applying variance structures (Zuur et al. 2009). For body mass, the best fit model yielded a combined variance structure (specified by "weights") accounting for different variation for underyearlings and yearlings, plus age-specific variation correlated with underyearling density (i.e., weights = varComb(varIdent(form ~ 1|age), varPower(form ~ TUY|age)). Using this variance structure and starting with the initial LMM model, nonsignificant terms ($P > 0.05$) were removed one at a time. In each step a reduced model without the term in question was compared with the same model including the term, following the procedure given in Zuur et al. (2009). Model performance was compared using log-likelihood ratios. The minimum adequate model was achieved when all nonsignificant parameters were removed. To compare models with different fixed effect structure, the model fits were based on the maximum likelihood (ML) method during the simplification procedure. Summaries and coefficients of the final models are based on the restricted likelihood (REML) method, which, unlike the ML method, gives an unbiased estimator of the variance (Crawley 2007; Zuur et al. 2009).

Whereas body mass is an accumulated measure of performance from time of hatching, the energy density is a more dynamic measure that changes over a shorter time span (Weber et al. 2003; Berg et al. 2006). Since there is a strong linear relationship between percent dry body mass (of wet mass) and energy density (Hartman and Brandt 1995), the dry mass to wet mass ratio (dm/wm) can be used as an indirect measure of performance. To test if density-dependent performance differed between underyearlings and yearlings, LMMs were constructed with this ratio as the response variable, body length and age- and species-specific densities as

Table 2. Parameter estimates of the fixed effects from the linear mixed effects model (LMM) best explaining variation in wet body mass and energy density (estimated by ratio of dry to wet body mass).

Response variable	Parameter	Coefficients (SE)	*t*
Wet body mass	(Intercept)	1.44 (0.13)	11.11
	Age (1)	1.52 (0.13)	11.42
	TUY	–0.16 (0.03)	–5.34
	Age (1) : TUY	0.08 (0.03)	2.42
Energy density	(Intercept)	–2.71 (0.06)	–43.8
	Age (1)	–0.03 (0.01)	–2.63
	Body length	0.29 (0.01)	22.5
	TUY	–0.015 (0.006)	–2.37

Note: Only significant parameters are included in the final models. Age (1) coefficients refer to yearlings. Sample size: $n = 714$. SE, standard error; TUY, trout underyearling density.

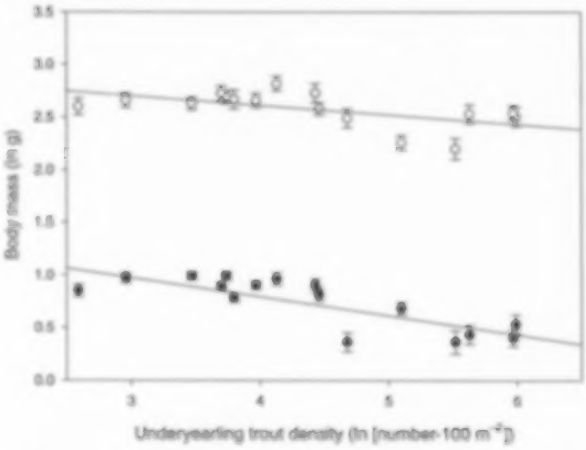
covariates, and age as a fixed factor. Body length was included as a covariate because of allometric relationships causing larger fish to have larger energy storage capacity. As for body mass, dm/wm and densities were ln-transformed. Initial exploration of the data indicated heteroscedasticity that was controlled for with appropriate variance functions (weights = varComb(varIdent(form ~ 1|age), varPower(form ~ TUY), varPower(form ~ TY)). A random structure with age nested in station (random = 1|[station/age]) was chosen for the energy density models, as this performed substantially better compared with a model with station only. This random structure allowed for station-specific random effects for the two age classes.

To test whether age- and species-specific densities affected the variation in body size within stations, we performed ANCOVAs with the standard deviation of body mass for each station as the response variable. Since density-dependent effects on average size would affect the coefficient of variation, standard deviation constitutes a less biased estimator. The effect of densities on variation in energy density was also analysed. To avoid the masking effect of the allometric relationship between body length and energy density, the residuals from a linear regression of dm/wm against length (both ln-transformed) were used as a response variable. Data exploration revealed differences in the spread of standard deviations for underyearlings and yearlings. For body mass, this was solved by ln-transforming body mass before calculating the standard deviation. For energy density residuals, the spread was largest for the smallest age class; hence, we applied generalized least squares models with a variance structure controlling for the difference in spread (i.e., weights = varIdent(form ~ 1|age)).

Results

For analyses of wet body mass, stepwise removal of nonsignificant interaction terms excluded all interactions except the one between age and underyearling trout density. Further stepwise removal of nonsignificant fixed effects resulted in a minimum adequate model given by age and trout underyearling density only. Hence, the body mass of both underyearlings and yearlings depended mostly on underyearling density, although the response (slope) was significantly less for the yearlings (Table 2; Fig. 2).

Fig. 2. Relationship between station-specific average wet body mass (ln g) ± standard error (SE) of brown trout underyearlings (solid circles) and yearlings (open circles) and underyearling density (ln (number·100 m^{-2})) in the River Gråelva. Separate regression lines are shown (underyearlings: $r^2 = 0.65$, $P < 0.001$; yearlings: $r^2 = 0.38$, $P = 0.008$).



For analyses of energy density (dm/wm), the final model included only age class and underyearling density as main effects (Table 2). The lack of significant interaction terms suggests that underyearling abundance reduces the energetic potential of yearlings to the same extent as that of underyearlings (Table 2). Estimating the best linear unbiased predictions of station-specific intercepts for a model without density as fixed factor (using the function "ranef" in R) reveals the density-dependent variation among stations (Fig. 3).

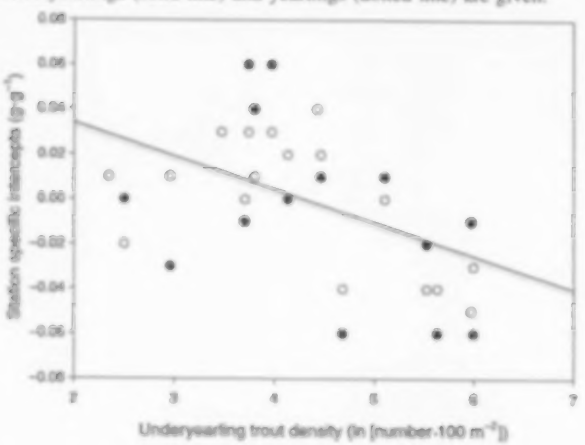
Tests based on station average performance confirmed the findings of a negative effect of underyearling density on both body mass and energy density (Table 3); however, no significant differences in the two age classes' response were found. In contrast with the LMM analysis, the ANCOVAs identified a significant negative effect of yearling salmon density on body mass (Table 3).

For underyearling trout, the variation in body mass increased with increasing abundance of their own age class, whereas density of yearlings had no significant effect (Table 4). For trout yearlings, the body mass variance also increased with the density of their own cohort; however, here underyearling density also had a significant, but negative, effect on the variation (Table 4). When testing for density effects on variation in energy density, after controlling for body length, no interaction terms were significant (Table 4). The variation in energy density increased with underyearling trout density while being reduced at increasing abundance of yearling trout (Table 4).

Discussion

In this study we found a negative effect of local underyearling trout density on body mass of both underyearling and yearling brown trout, whereas the impact of yearling trout density and underyearling and yearling salmon density were nonsignificant. Since body mass is an accumulated

Fig. 3. Estimated best linear unbiased predictions of station-specific intercepts (ln g·g^{-1}) from a linear mixed effect model of dry to wet body mass ratio against body length for underyearling (solid circles) and yearling (open circles) brown trout plotted against underyearling density (ln (number·100 m^{-2})). Linear regression lines for underyearlings (solid line) and yearlings (dotted line) are given.



measure of growth from hatching to present, the body mass of yearlings will not only depend on growth during the current season, but also on growth rates experienced the previous year. We therefore analysed the data with respect to energy density measured in terms of dry to wet mass ratio while controlling for body length. Energy or lipid levels in salmonid juveniles constitute a more appropriate measure of current growth performance because it changes with food availability over short time scales (Weber et al. 2003), and it has previously been shown not to be correlated with body size during early spring in juvenile brown trout (Berg and Bremset 1998). Yet, yearling energy density showed a similar response, with no influence of yearling density, but a negative density effect of underyearlings.

The strong influence of underyearling local density on their own performance suggests a limited scope for movement and homogenization of densities, as shown in previous studies (Einum et al. 2006, 2008*a*). In contrast, spatial variation in yearling body mass or energetics did not appear to depend on their own density. This finding suggests that yearlings are more efficient in homogenizing densities according to their own effects on the local growth potential. However, the yearlings do not appear to consider the density of underyearlings when distributing themselves, even if they appear to have a negative effect on their growth performance. This interpretation is also supported by the lack of a negative correlation between densities of the two age classes (Pearson's $r = 0.42$, $P = 0.09$, degrees of freedom = 15), suggesting that older fish distribute themselves according to other factors than growth potential per se. Spatial variation in the amount of suitable physical habitats may be more likely to shape the distribution of yearlings. For example, previous studies have shown that the local carrying capacity of yearling and overyearling Atlantic salmon to a large extent can be related to shelter availability (Finstad et al. 2009). Furthermore, under experimental conditions juvenile coho salmon (*Oncorhynchus kisutch*) preferred a patch with

Table 3. Parameter estimates for the effect of specific densities on body mass and energy density residuals given by analyses of covariance (ANCOVAs).

Response variable	Parameter	Coefficients (SE)	t	P
Wet body mass	(Intercept)	1.12 (0.15)	7.53	<0.001
	Age (1)	1.84 (0.05)	38.3	<0.001
	TUY	−0.13 (0.02)	−5.31	<0.001
	TY	0.10 (0.05)	1.84	0.08
	SUY	0.03 (0.02)	1.35	0.19
	SY	−0.06 (0.03)	−2.10	0.04
Energy density residuals	(Intercept)	0.051 (0.034)	1.51	0.14
	Age (1)	−0.014 (0.011)	−1.27	0.22
	TUY	−0.013 (0.006)	−2.35	0.03
	TY	0.010 (0.012)	0.82	0.42
	SUY	0.011 (0.005)	2.09	0.05
	SY	−0.012 (0.007)	−1.77	0.09

Note: All interaction terms were nonsignificant. TUY and TY refer to trout underyearling and yearling density, respectively; SUY and SY refer to salmon underyearling and yearling density, respectively. Age (1) coefficients refer to yearlings. Sample size: $n = 34$. SE, standard error.

Table 4. Parameter estimates for the effect of specific densities on the standard deviations in body size given by analysis of covariance (ANCOVA) and for standard deviations in energy density after controlling for body length given by generalized least squares.

Response variable	Parameter	Coefficients (SE)	t	P
Wet body mass	(Intercept)	−1.04 (0.24)	−4.35	<0.001
	Age (1)	1.80 (0.33)	5.46	<0.001
	TUY	0.14 (0.04)	3.43	0.002
	TY	−0.04 (0.08)	−0.55	0.59
	SUY	−0.07 (0.04)	−1.93	0.065
	SY	0.00 (0.04)	−0.06	0.96
	Age (1) : TUY	−0.24 (0.06)	−4.16	<0.001
	Age (1) : TY	0.43 (0.11)	4.06	<0.001
	Age (1) : SUY	0.20 (0.05)	3.75	<0.001
Energy density residuals	(Intercept)	0.061 (0.004)	6.15	<0.001
	Age (1)	−0.010 (0.004)	−2.38	0.026
	TUY	0.004 (0.002)	2.47	0.020
	TY	−0.012 (0.003)	−3.50	0.002
	SUY	0.001 (0.001)	0.59	0.56
	SY	0.003 (0.002)	1.61	0.12

Note: Only significant interaction terms are included. TUY and TY refer to trout underyearling and yearling density, respectively; SUY and SY refer to salmon underyearling and yearling density, respectively. Age (1) coefficients refer to yearlings. Sample size: $n = 34$. SE, standard error.

cover but little food compared with a patch with more food without cover (Grand and Dill 1997). Because shelter availability influences growth potential (Finstad et al. 2007), a spatial distribution of yearlings that matches the availability of shelters will reduce the spatial density dependence in growth that they impose upon their own age class.

Although mean body mass of yearlings did not depend on their own density, increasing size variance with increasing density indicates asymmetric effects depending on their size. The result suggests that body size of the largest individuals increased with yearling density, supporting the assumption of a distribution according to resource abundance, whereas the smaller individuals were negatively affected by

intracohort density. The age-specific response to density observed for variation in body mass was not found when analysing variation in dm/wm. This might indicate that effects of competition have changed (i.e., the observed pattern of size-at-age is a result of competition earlier in life). However, large variation in estimated energy density within each site (coefficient of variation = 6.9 ± 3.4) reduces the power of tests for interactions between age and age-specific density.

The finding that underyearlings did not affect energy density of their own age class more than the energy density of the older age class suggests that underyearling density has a similar effect on yearling and underyearling habitat quality in terms of growth potential. Few studies have addressed the question of intercohort competition in stream-dwelling salmonids, and those that do have focused on negative effects of older fish on underyearlings (Greenberg et al. 1997; Nordwall et al. 2001; Kaspersson and Höjesjö 2009). Our study, on the other hand, suggests the possibility for the reverse effect, that is, underyearling trout density can influence performance of older age classes. In stream salmonids, body size has a large effect on competitive abilities during interference competition (e.g., Bachman 1984; Elliott 1990), and underyearlings rarely attack older juveniles intruding into their territories (Steingrímsson and Grant 2008). Thus, interference competition and dominance from underyearlings seem unlikely to be responsible for our observations. However, vigilance and defence of territories may still cause increased metabolic costs and reduced feeding efficiency, as yearlings behave aggressively towards underyearlings and increase activity as their density increases (Vehanen et al. 1999; MacLean et al. 2005; Kaspersson et al. 2010). Alternatively, exploitative competition causing reduced food availability for older cohorts with increasing density of underyearlings may be responsible for our observation. Intercohort foraging competition with such negative effects of smaller sized fish has been observed in Arctic char (*Salvelinus alpinus*) feeding in lakes (Byström and Andersson 2005), where interference competition appears unlikely. Exploitative competition has also been suggested to cause reduced growth at low densities in stream salmonids (<1 individual·m^{-2}; Grant and Imre 2005; Imre et al. 2005; but see Ward et al. 2007). Thus, reduced energetic performance of older cohorts with increasing abundance of underyearlings can be caused by reduced food availability, increased metabolic costs, reduced foraging efficiency, or a combination of these mechanisms.

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Incorporating climate changes into population dynamic modelling: an individual-based modelling approach for lobster

Yi-Jay Chang, Chi-Lu Sun, Yong Chen, Yuying Zhang, and Su-Zan Yeh

Abstract: One of the most challenging issues in fisheries management is the evaluation of the effects of fishing in the context of a changing environment. Using the pronghorn spiny lobster (*Panulirus penicillatus*) fishery off the eastern coast of Taiwan as an example, we developed an individual-based model (IBM) that is capable of describing the temperature-dependent life history processes and fishery practices for the spiny lobster. We then used the model to evaluate potential impacts of increased ocean temperature on the estimation of mortality-based biological reference points for fisheries management. We demonstrate that a warming temperature would increase the yield-per-recruit and eggs-per-recruit values and consequently reduce the risk of overexploitation under the current exploitation level. However, there is likely a high risk of overexploitation in the long term if higher temperatures induce extra-high natural mortality. The evaluation of effectiveness of size regulations suggests that increasing minimum legal size is proposed as a good candidate measure to reduce the risk of overexploitation for pessimistically unfavorable environmental conditions. This study suggests that an explicit incorporation of the relationships between environmental variables and biological processes can greatly improve fisheries assessment and management.

Résumé : Un des plus importants défis dans la gestion des pêches est l'évaluation des effets de la pêche dans le contexte d'un environnement en changement. Prenant comme exemple la pêche de la langouste fourchette (*Panulirus penicillatus*) au large de la côte ouest de Taiwan, nous mettons au point un modèle basé sur l'individu (IBM) capable de décrire les processus du cycle biologique reliés à la température et les pratiques de pêche pour la langouste fourchette. Le modèle nous sert ensuite à évaluer les impacts potentiels de la température croissante de l'océan sur l'estimation des points de référence biologiques basés sur la mortalité pour la gestion de la pêche. Nous démontrons qu'une température croissante ferait augmenter les valeurs du rendement par recrue et des œufs par recrue et réduirait ainsi le risque de surexploitation au niveau actuel d'exploitation. Cependant, il y a vraisemblablement un risque élevé de surexploitation à long terme si la température plus chaude entraîne une mortalité naturelle particulièrement forte. L'évaluation de l'efficacité des règlementations de la taille laisse penser qu'un accroissement de la taille légale minimale serait une bonne mesure à retenir afin de réduire le risque de surexploitation advenant des conditions environnementales particulièrement défavorables. Notre étude indique que l'incorporation explicite des relations entre les variables environnementales et les processus biologiques peut grandement améliorer les évaluations et la gestion de la pêche.

[Traduit par la Rédaction]

Introduction

Understanding the effects of fishing pressure and environmental variability on the dynamics of exploited populations is critical for fishery management (Glantz 1992). Many commercially important fish populations, such as top predator species in an ecosystem, have been impacted in the past several decades as a consequence of fisheries industrialization (Hutchings and Reynolds 2004; Worm et al. 2009), although the level of depletion identified in various studies differed (e.g., Sibert et al. 2006). While fishing is one of the factors contributing to the decline of many fish populations, a deteriorating environment related to climate change combined with overfishing may result in fishing down below the level of minimum population biomass, such as Pacific sardine (*Sardinops sagax caerulea*) around California and Atlantic cod (*Gadus morhua*) around Newfoundland (Hilborn et al. 2003; Lees et al. 2006). Whereas the processes affecting the stocks dynamics are increasingly understood now, the consequences of environmental changes for fisheries management are far less evident. This includes doubts about the adequacy of biological reference points (BRPs) currently used and the need to revise BRPs given

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Y.-J. Chang, C.-L. Sun,[1] and S.-Z. Yeh. Institute of Oceanography, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan.
Y. Chen and Y. Zhang, School of Marine Sciences, University of Maine, Orono, ME 04469, USA.

[1]Corresponding author (e-mail: chilu@ntu.edu.tw).

that environmental changes may affect stock productivity (e.g., American lobster (*Homarus americanus*) fisheries: Fogarty and Gendron 2004; North Sea demersal fisheries: Cook and Heath 2005).

Lobsters, large marine crustaceans (Decapod), play important roles as a predator and prey in marine benthic ecosystems and in worldwide commercial and artisanal fisheries (Phillips and Kittaka 2000; Phillips 2006). Statistics of the Food and Agriculture Organization of the United Nations (2006) indicate that 251 000 tons of lobsters were landed in 2005 from marine systems with a landing value of over US$2.346 billion. The complex life histories and fisheries processes of crustaceans offer unique challenges for stock assessment modelers (Bunnell and Miller 2005). For example, the growth of crustaceans is molt-dependent (stepwise growth) in contrast with the continual growth of finfish. It is difficult to determine the age of crustaceans because of a lack of calcified tissues (e.g., otoliths, spines, and vertebrae), which have been used in other taxa to estimate age, although this problem may be partly overcome by lipofuscin ageing (Sheehy et al. 1998). The complex life cycle of crustaceans make their population dynamics more likely to be influenced by environmental variability. For example, the recruitment of lobsters was influenced by the degradation of preferred settlement habitat of postlarval pueruli (macroalgae-covered, hard-bottom habitat) for *Panulirus argus* (Butler 2003) and the variation of ocean current strength through the prolonged larval stages (9–11 months) for *Panulirus cygnus* (Caputi et al. 2010). However, traditional fishery population models typically perform poorly in handling these dynamic processes and incorporating individual variability (Smith and Addison 2003).

Since the 1970s, individual-based models (IBMs) (Huston et al. 1988; DeAngelis and Gross 1992) have been increasingly used to model complex population dynamics based on individual characteristics (variability), rather than mimicking the average population patterns with mathematical formulations. IBMs follow the life history process of each individual in a population; the survival, growth, and reproduction of which are affected by individual characteristics; local habitats; and other stochastic processes (Uchmanski and Grimm 1996). IBMs are widely used to study the effects of fishing and environmental changes on the dynamics of marine populations. For example, Hobday and Tegner (2002) used an IBM to investigate the extinction risk of two red abalone (*Haliotis rufescens*) populations under different environmental scenarios in the Southern California Bight, USA. Butler (2003) used a spatially explicit IBM to evaluate recruitment variations with environmental changes of *P. argus*.

Per-recruit analyses are commonly used to estimate the yield, spawning biomass, or egg production an average recruit is expected to produce over its lifespan at a specific exploitation rate, taking into account the trade-off between growth and mortality (Ricker 1975). The analysis is often used to develop BRPs that are commonly used in the management of crustacean fisheries (Annala and Breen 1989; Chen and Wilson 2002). In this study, using the pronghorn spiny lobster (*P. penicillatus*) off the eastern coast of Taiwan as an example, we developed an IBM approach to incorporate variability among individuals and environmental effects (e.g., temperature change) into the per-recruit analyses and estimate mortality-based BRPs (F_{BRPs}) and their uncertainties.

Climate models of the effect of climate change project a general warming pattern of sea-surface temperature (SST; 1–3 °C) around Taiwan waters by the 2030s (Intergovernmental Panel on Climate Change 2007); however, there is much greater uncertainty in general physical oceanography. Temperature is known to be the most important environmental factor to affect the biology of crustaceans (Hartnoll 2001). To examine how increased ocean temperature may affect the future lobster stock productivity and its associated management implications, we developed a model to evaluate potential impacts of temperature change on the population dynamics, estimates of F_{BRPs}, and risk of overexploitation in the long term. The effectiveness of a set of minimum legal size regulations was evaluated for the risk of overexploitation under the defined temperature regimes. The implications for the management of crustacean fisheries under an increased temperature are discussed.

Materials and methods

Development of an IBM

We developed an IBM that quantifies the population life history and fishery processes of *P. penicillatus* off the eastern coast of Taiwan (see Chang et al. 2007, 2009 for the life history and fishery) by monitoring the daily fate of each individual. The individual life cycle of *P. penicillatus* after being recruited to the fishery was developed (Fig. 1). Because life history processes of males are simpler than those of females, and the females were largely determining the reproductive potential (assume no male limitation for this both-sexes fishery compared with sex-specific fisheries, e.g., king crab (*Paralithodes brevipes*) fishery off eastern Hokkaido; Sato et al. 2007), only female lobsters were considered in the following analysis. At each time step, each individual has a probability of being caught in the fishery, dying of natural mortality and (or) molting mortality, growing, maturing, and becoming an eggs-carrying female (Fig. 1). The model was carried out by expressing various model components as random Bernoulli trials (Chen et al. 2005). For example, rather than calculating the number of spiny lobsters that survive natural mortality by

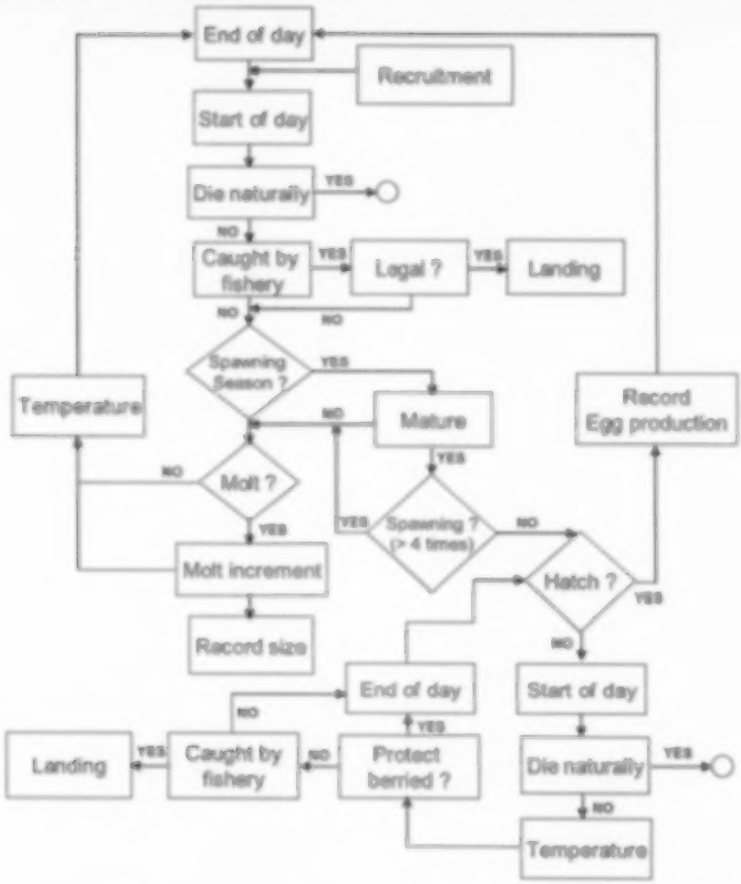
$$N_{t+1} = N_t e^{-M}$$

where M is the instantaneous rate of natural mortality, we simulated the natural mortality acting on each individual spiny lobster as follows: if $U(0,1) \leq 1 - e^{-M}$, an individual would die and the number of spiny lobsters that survive natural mortality is updated as

$$N_{t+1} = N_t - 1$$

where $U(0,1)$ is a uniformly distributed random number between 0 and 1. The model's time step is 1 day to facilitate addressing questions such as the effects of daily temperature variations. Because *P. penicillatus* can spawn multiple times throughout the year, before the simulation we determined the temporal pattern of recruitment at the beginning of the time series for the analysis. Based on the monthly proportion of berried (eggs-carrying) females, we simply estimated

Fig. 1. Flowchart for the individual-based model (IBM) of the spiny lobster, *Panulirus penicillatus*. The management measures (size regulations and protecting berried females) are also included in the IBM.



the seasonal proportions of lobsters recruiting into the fishery after reaching 35 mm by simulating the impacts of daily water temperature on the egg incubation duration and growth of juvenile lobsters after settlement (>3 mm carapace length (CL)). No temperature-dependent development rate for the larval phase and mortality before recruitment were assumed in this presimulation (burn-in) phase of simulation. Therefore, lobsters were modeled as being recruited into the fishery at a size drawn from a normal distribution (mean = 36 mm CL and standard deviation = 1.5 mm) following one molt by different seasonal proportions: 13% of annual recruitment occurring in summer, 20% in spring, 50% in fall, and 17% in winter based on the results of burn-in phase to simplify the model. The detailed parameterization of each module of the lobster IBM is presented below.

Growth module

To describe the stepwise patterns of changes in the size of the crustaceans, we quantified the two components that determine the growth process — (*i*) molt increment (MI, the growth increment per molt) and (*ii*) intermolt period (IP, the time that elapses between the two consecutive molts) — to form a complete description of growth in terms of size versus time. For tropical spiny lobster (e.g., *P. penicillatus*), the individuals molt independently and asynchronously throughout the year (Wahle and Fogarty 2006). In the model, lobsters grew discontinuously by molting, the magnitude and frequency of which were governed by temperature and lobster size (Hartnoll 2001; Wahle and Fogarty 2006). The MI and IP data of *P. penicillatus* were obtained from rearing studies of Plaut and Fishelson (1991) and Huang (2009). A previous rearing experiment suggested that the MI between different size groups of *P. penicillatus* is not significantly related to temperature (analysis of covariance, ANCOVA; $p > 0.05$) (Huang 2009). Furthermore, the effect of temperature on the MI of crustaceans is variable (Hartnoll 1982); thus, the only temperature-dependent IP was considered in this study. To include temperature-dependent variation in IP, the chronological IP was changed to the physiological degree-day ($IP_{degree\text{-}day}$) using the degree-day approach (Wainwright and Armstrong 1993; Brylawski and Miller 2006):

$$(1) \quad IP_{degree-day} = (T - T_{min}) \times IP$$

where T is the daily temperature, and T_{min} is the physiological minimum temperature (4.5 °C) that was back-calculated

using the relationship between the development rate and temperature (Huang 2009). Because previous studies of *P. penicillatus* suggested that there was no significant difference in IP between different size classes (Ebert and Ford 1986; Plaut and Fishelson 1991; Huang 2009), instead of modelling changes in $IP_{degree\text{-}day}$ with size (Mauchline 1977; Wainwright and Armstrong 1993; Wahle and Fogarty 2006), we used a normal distribution ($IP \sim N(\mu_{IP}, \sigma^2_{IP})$) to describe the variability in $IP_{degree\text{-}day}$ of each molt for each individual.

The temperature-dependent stepwise growth curve was simulated by comparing a random number q ($q \sim U(0,1)$) and the probability of molting at a given $IP_{degree\text{-}day}$ ($P(IP_{degree\text{-}day})$; see Chen and Kennelly 1999; Brylawski and Miller 2006): if $q < P(IP_{degree\text{-}day})$, then accept that one molt occurred, whereas if $q > P(IP_{degree\text{-}day})$, then accept that a molt did not take place. $IP_{degree\text{-}day}$ accumulates by daily temperature, which is repeated until $P(IP_{degree\text{-}day}) > q$, and we assume one molt occurred. The MI is randomly drawn from a normal distribution of given size class ($MI(CL) \sim N(\mu_{CL}, \sigma^2_{CL})$) (Table 1). To prevent a small proportion of lobsters from growing indefinitely, the lobsters were assumed to have a maximum age of 30 years in the IBM based on a study using neurolipofuscin for aging the tropical spiny lobster (*P. argus*, Maxwell et al. 2007).

Reproduction module

The proportion of mature lobsters at a given CL, $P_m(CL)$, is described by a logistic function that represents the probability of size-at-functional maturity (Table 1). Studies suggested that *P. penicillatus* were actively reproductive throughout the year, with a major spawning peak in the summer as a result from higher temperature (Juinio 1987; Plaut 1993; Chang et al. 2007). Therefore, we simulated the spawning event of an individual lobster at a given size when the temperature is higher than 25 °C according to our field experimental data (Chang et al. 2007) and $P_m >$ random number q. We modeled that the small females (<60 mm CL) could spawn at least two times and large lobsters (≥60 mm CL) could spawn up to four times between two consecutive molts during the spawning season. In the model, an individual has a chance to molt following hatching of the carried eggs; the molt process restarts to accumulate the degree-days needed for molting. If the individual does not molt, then it would have a chance to successively mating and brooding when it satisfies the above constraints. A shorter incubation period at higher temperatures was observed in various tropical spiny lobsters (Chittleborough 1976; Plaut 1993), and embryos generally required a total of 750 degree-days to hatch (30 days at 25 °C). In the model, the fecundity (brood size) changes with lobster size by a power function (Table 1) and is assumed to stay constant for successive spawners without changes in their sizes.

Mortality module

Studies suggested that temperature would directly affect natural mortality (M) through metabolic rate or indirectly affect M via life history optimization (Brown et al. 2004; Charnov and Gillooly 2004). In this study, a list of estimates of L_∞ and K of von Bertalanffy growth function (VBGF) and annual mean SST of the associated region for various marine lobsters were obtained from literature (Appendix Table A1). Assuming K was an index of physiological activity (metabolic rate), a significant linear relation between K and temperature (T) was found as $K = 0.0239\,T - 0.2988$ (F test, $p < 0.05$). The relationship between L_∞ and T is not significant; therefore, we substituted the direct proportion ($K \propto 0.0239 \times T$) into Pauly's (1980) empirical function to estimate the temperature-dependent mortality. For example, the estimated M for the current temperature regime is 0.46 year^{-1} (L_∞ = 105.6 mm CL, K = 0.22 year^{-1}, and annual mean SST = 26.4 °C), and M increases to 0.50, 0.54, and 0.58 year^{-1} with increased annual mean SST 1–3 °C (respectively) at the fixed L_∞ value and the K–temperature proportional relationship based on the Pauly's function.

M was assumed to occur before instantaneous fishing mortality (F). The annual probability of surviving natural mortality was e^{-M}. Thus, the daily probability of natural mortality was equal to $1 - e^{-M/365}$, and the same was done for F (i.e., $1 - e^{-F/365}$). Each individual lobster in the simulation was characterized as a super-individual in that each represented some larger number of individuals (an internal amount, a) in the population (sensu Scheffer et al. 1995). The model simulated each super-individual by having the super-individual die (then a becomes zero). The number of deaths (d_t) on day t for each super-individual is a binomial trial and approximates a normal distribution with mean of $a_t \times p$ and a standard deviation of $[a_t p(1 - p)]^{0.5}$ when $a_t \times p > 5$, and p is the daily probability of dying because of M or F (Scheffer et al. 1995; Bunnell and Miller 2005). Studies suggest that recently molted lobsters might be more vulnerable to predation (or cannibalism) and other sources of mortality; thus, the probability of dying because of M for a softshell lobster prior to hardening (3 days duration) was considered twice the probability of that for a hardshell lobster (Huang 2009).

Evaluating the performance of the IBM

To evaluate the performance of the IBM, we compared seasonal size distributions of landed *P. penicillatus* generated from our simulation model based on the parameterization described above with the observed seasonal size distributions of landings in Taitung waters in 2003–2004. The likelihood function for the multinomial length–frequency data was used to evaluate the performance of fitting (eq. 2.2 of Fournier et al. 1990). Estimated values and variations in F_{BRPs} may depend on the number of individuals of a cohort chosen at the beginning of the simulation. For each super-individual, the life history processes of all individuals it represents (i.e., a = 10 000 lobsters) were the same in the model. Thus, the effective sample size is one in this case. We evaluated the effect of different effective sample sizes on the BRP estimation before further analyses.

Estimation of BRPs

For each super-individual, the yield-per-recruit (YPR) and eggs-per-recruit (EPR) values were determined by dividing the total catch and egg production by the initial recruitment of year class, respectively, with respect to different fishing mortalities. Median values of the results derived from 300 super-individuals were used to obtain YPR and EPR curves for estimating F_{BRPs} (e.g., F_{max}, $F_{0.1}$, and $F_{x\%}$). Previous

Table 1. List of models and parameters used in this study.

Description	Model and parameter	Parameter value	Reference
Model for body mass (g) of females at a given carapace length, BM(CL)	$BM(CL) = a_1(CL)^{b_1}$	$a_1 = 0.0013$ and $b_1 = 2.89$	Chen 2005
Molt increment (mm) for females of different CL groups follows normal distributions	$MI(CL) \sim N(\mu_{CL}, \sigma^2_{CL})$	CL = 30–40, $\mu = 1.51$, $\sigma = 0.27$ CL = 40–50, $\mu = 2.07$, $\sigma = 0.31$ CL = 50–60, $\mu = 1.03$, $\sigma = 0.13$ CL = 60–70, $\mu = 0.54$, $\sigma = 0.12$ CL = 70–80, $\mu = 0.25$, $\sigma = 0.21$ CL = 80–90, $\mu = 0.40$, $\sigma = 0.92$ CL = 90–100, $\mu = 0.20$, $\sigma = 0.7$ CL > 100, $\mu = 0.23$, $\sigma = 0.47$	Plaut and Fishelson 1991; Huang 2009
Intermolt period (degree-days) for females follows normal distributions	$IP \sim N(\mu_{IP}, \sigma^2_{IP})$	$\mu_{IP} = 2971.72$ and $\sigma_{IP} = 1066.98$	Huang 2009
Physiological minimum temperature (°C) for growth	T_{min}	4.5	Derived from data of Huang 2009
Model for proportion of mature females (%) at a given carapace length, $P_m(CL)$	$P_m(CL) = \frac{1}{1+e^{(b_2+a_2CL)}}$	$a_2 = -0.11$ and $b_2 = 66.63$	Chang et al. 2007
Model for fecundity (number of eggs) of females at a given carapace length, fec(CL)	$fec(CL) = a_3CL^{b_3}$	$a_3 = 2.4\times10^{-3}$ and $b_3 = 4.18$	Chang et al. 2007
Natural mortality rate (year^{-1})	M	0.46	Chang et al. 2009
Current fishing mortality rate (year^{-1}), F_{cur}	$F_{cur} \sim N(\mu_F, \sigma^2_F)$	$\mu_F = 0.37$ and $\sigma_F = 0.06$	Chang et al. 2009

studies suggest that F_{max} was poorly defined for the Taitung lobster fishery because the relationship between yield and F was asymptotic when M was high relative to growth (Chang et al. 2009). Another biological reference point ($F_{0.1}$), which is widely used as a target fishing mortality level, is defined as the fishing mortality rate at which the marginal increase in YPR is 10% of that at $F = 0$ (Hilborn and Walters 1992). We calculated the derivative of YPR with respect to fishing mortality rate to estimate $F_{0.1}$ iteratively from the following equation:

$$(2) \qquad \frac{\partial(\mathrm{YPR}_F)}{\partial F}\Big|_{F=F_{0.1}} = 0.1\frac{\partial(\mathrm{YPR}_F)}{\partial F}\Big|_{F=0}$$

Another common BRP based on EPR is the value $F_{x\%}$, which is defined as the instantaneous rate of fishing mortality that reduces EPR to x% of the level that would exist with no fishing (Chen and Wilson 2002). For example, $F_{40\%}$ can be estimated as

$$(3) \qquad \mathrm{EPR}_F\big|_{F=F_{40\%}} \approx 0.4\mathrm{EPR}_F\big|_{F=0}$$

We estimated $F_{10\%}$, $F_{20\%}$, $F_{30\%}$, and $F_{40\%}$ from the EPR analysis. In this study, YPR and EPR values were calculated under various fishing mortalities (step size 0.001) in advance, then F_{BRP}s were estimated from the systematical numerical search by approximating the above F_{BRP} definitions (eqs. 2–3). Furthermore, the IBM was repeatedly run 500 times with different random number seeds to estimate the uncertainty of the F_{BRP}s.

Increased ocean temperature and management implications

To evaluate the potential impacts of increased ocean temperature on the lobster population dynamics, we considered the expected changes in SST in the eastern waters of Taiwan inhabited by lobsters (Intergovernmental Panel on Climate Change 2007). Because *P. penicillatus* inhabit shallow water (1–4 m), we assumed that temperature changes would result in changes in key lobster habitats in the future. A pair of simulations in which each temperature scenario was presented with and without presumed temperature effects on M was considered to evaluate the impacts of temperature change on the stock productivity (Table 2). Furthermore, a scenario of presumed extra-high mortality (i.e., 0.69 year^{-1}; 1.5-fold the current M) at the highest temperature regime (increased 3 °C) was considered.

Because the BRPs derived from the scenarios with and without temperature changes are not comparable, the slopes of YPR curves and the EPR values of scenarios 1T–3THM considered were compared with those estimated for scenario CT (i.e., the level of productivity without temperature changes) to evaluate the impacts of temperature changes. $F_{0.1}$ of the YPR analysis at an increased T temperature scenario was estimated as

$$(4) \qquad \frac{\partial(\mathrm{YPR}_F^{+T})}{\partial F}\Big|_{F=F_{0.1}} = 0.1\frac{\partial(\mathrm{YPR}_F^{cur})}{\partial F}\Big|_{F=0}$$

where YPR_F^{+T} and YPR_F^{cur} are the yield-per-recruit values at fishing mortality for the scenario of temperature increase (e.g., scenarios 1T–3THM) and current temperature regime (i.e., scenario CT), respectively. For the EPR analysis, $F_{40\%}$ at an increased temperature scenario was estimated as

$$(5) \qquad \frac{\mathrm{EPR}_F^{+T}}{\mathrm{EPR}'}\Big|_{F=F_{40\%}} \approx 0.4\frac{\mathrm{EPR}_F^{+T}}{\mathrm{EPR}'}\Big|_{F=0}$$

where EPR_F^{+T} is the eggs-per-recruit value at F for the scenario of temperature increase (e.g., scenarios 1T–3THM) and EPR′ is the eggs-per-recruit value at $F = 0$ for the current temperature regime (i.e., scenario CT).

Table 2. Simulation scenarios considered in evaluating the impacts of increased ocean temperature on the estimation of biological reference points based on the prediction of future temperatures from the Intergovernmental Panel on Climate Change (2007).

Scenario	Average annual SST (°C)	Natural mortality ($year^{-1}$)
Current temperature regime (years 2003–2004)		
CT	26.4	0.46
Increased temperature would effect growth and reproduction only		
1T	27.4	0.46
2T	28.4	0.46
3T	29.4	0.46
Increased temperature would effect growth, reproduction, and mortality		
1TM	27.4	0.49
2TM	28.4	0.52
3TM	29.4	0.55
Increased temperature would effect growth, reproduction, and introduce extra mortality		
3THM	29.4	0.69

The status of a fishery is often determined by comparing an indicator reference point (e.g., F_{cur}) with a management reference point (e.g., $F_{0.1}$) for long-term sustainability (Chen and Wilson 2002). We determined the biological risk of overfishing as a probability ($P(F_{cur} > F_{BRP})$) based on the uncertainty in both estimates of F_{cur} (from Chang et al. 2009) and the IBM-based F_{BRP} following a composite risk analysis (Jiao et al. 2005):

$$(6) \qquad P(F_{cur} > F_{BRP}) = 1 - \sum_{-\infty}^{+\infty} G(x)f(x)\Delta x$$

where $G(x)$ is the empirical cumulative distribution function for F_{cur}, $f(x)$ is the empirical probability density function for F_{BRP}, and Δx is the mortality step size for F_{BRP}.

To assess the effectiveness of different potential management measures for reducing the risk of overexploitation under unfavorable environmental conditions (i.e., high temperature introduced extra-high mortality; scenario 3THM; Table 2), a set of minimum legal sizes (MLS; 40, 45, 50, and 55 mm CL; $F = 0$ when CL < MLS) was used to evaluate the composite risk of overexploitation and examine implications for fisheries management.

Results

Evaluation of the IBM performance

Generally, model fitting to the seasonal length–frequency data given the literature-derived parameters (see Table 1) can mimic the length–frequency data (Fig. 2; log-likelihood = 1152.01), suggesting that the IBM parameterization described in this study could realistically capture the dynamics of the targeted lobster population. However, the observed size distributions were well fitted by the predicted size distributions (Fig. 2; log-likelihood = 1199.53) when the mortality was lower (M of 0.29 $year^{-1}$, F of 0.22 $year^{-1}$) based on the grid searches of F and M (both from 0.2 $year^{-1}$ to 0.5 $year^{-1}$ with step 0.01). In this study, we chose an effective sample size of 300 because it was sufficient to yield stable estimates of the median and 95% confidence intervals of F_{BRP}s (Fig. 3). Thus, we used three million individuals to simulate the population dynamics.

YPR analysis

For each simulation scenario, the median YPR (g) of 500 runs increases asymptotically with increased fishing pressure (Fig. 4). If temperature affects growth and reproduction only, the results of median YPR curves increased with increasing temperatures (scenarios CT–3T). However, the median YPR curves decreased with increasing temperatures if increased temperature also introduces mortality (scenarios 1TM–3TM). Furthermore, the median YPR curve of the high temperature extra-high mortality scenario (scenario 3THM) was relatively low. This suggests that a higher temperature had a higher yield potential when mortality was independent of temperature, but the yield potential decreased with the temperature-dependent mortality. The indefinite increase in the YPR curves for all scenarios suggests that F_{max} was poorly defined in this study. The empirical distribution of $F_{0.1}$ for the years 2003–2004 (scenario CT) had a median value of 0.46 $year^{-1}$ (with a coefficient of variation (CV) of 4.34%). Comparing the results of scenario CT with those of scenarios 1T–3T and scenarios 1TM–3THM suggests that the median value of the $F_{0.1}$ distribution was not sensitive to changes in temperature or mortality (Table 3). The composite risk of overexploitation ($P(F_{cur} > F_{0.1})$) in 2003–2004 was 0.069. Because there was only a slight difference in $F_{0.1}$ among different scenarios, the risks of overexploitation were similar between scenarios (0.069, 0.070, 0.049, 0.042, 0.036, 0.021, 0.022, and 0.030 for scenarios CT to 3THM, respectively). However, the mean YPR values at $F_{0.1}$ (i.e., 38, 39, 41, 42, 35, 34, 32, and 26 g for scenarios CT–3THM, respectively) significantly differed between scenarios (analysis of variance, ANOVA, $p < 0.01$).

EPR analysis

Relationships between EPR values and fishing mortality rates varied with different scenarios (Fig. 4). The median EPR curves increased with temperature through increased spawning frequency of the lobster for the temperature-independent mortality scenarios (scenarios CT–3T). For the temperature-dependent scenarios (scenarios 1TM–3TM), the median EPR curve decreased with increasing temperature at lower fishing mortality levels, but median EPR curve was not sensitive to the temperature changes at higher fishing mortality levels. However, the EPR curve was relatively low for the high temperature extra-high mortality scenario (scenario 3THM). Median values of empirical distributions of F_{BRP}s in the years 2003–2004 were 0.63 (with a CV of 0.65%), 0.39 (1.18%), 0.27 (0.37%), and 0.19 (0%) $year^{-1}$ for $F_{10\%}$, $F_{20\%}$, $F_{30\%}$, and $F_{40\%}$, respectively (Table 3). For each reference level, the median value of the F_{BRP}s distribution increased with temperature (scenarios CT–3T), but F_{BRP}s were relatively stable among different temperature-dependent mortality scenarios (i.e., scenarios 1TM–3TM; Fig. 5, Table 3) resulting from the balance between the enhanced reproductive activity and increased mortality. Furthermore, the median value of F_{BRP}s for the extra-high

Fig. 2. Validation of the individual-based model (IBM). Comparison of the observed seasonal size distributions of the catch (gray bars) of the female spiny lobster, *Panulirus penicillatus*, off the eastern coast of Taiwan with simulated seasonal size distributions of the catch (black lines) from the IBM based on published parameters (*a*) and a scenario of lower mortality rates (*b*) (natural mortality of 0.29 year^{-1}, fishing mortality of 0.22 year^{-1}).

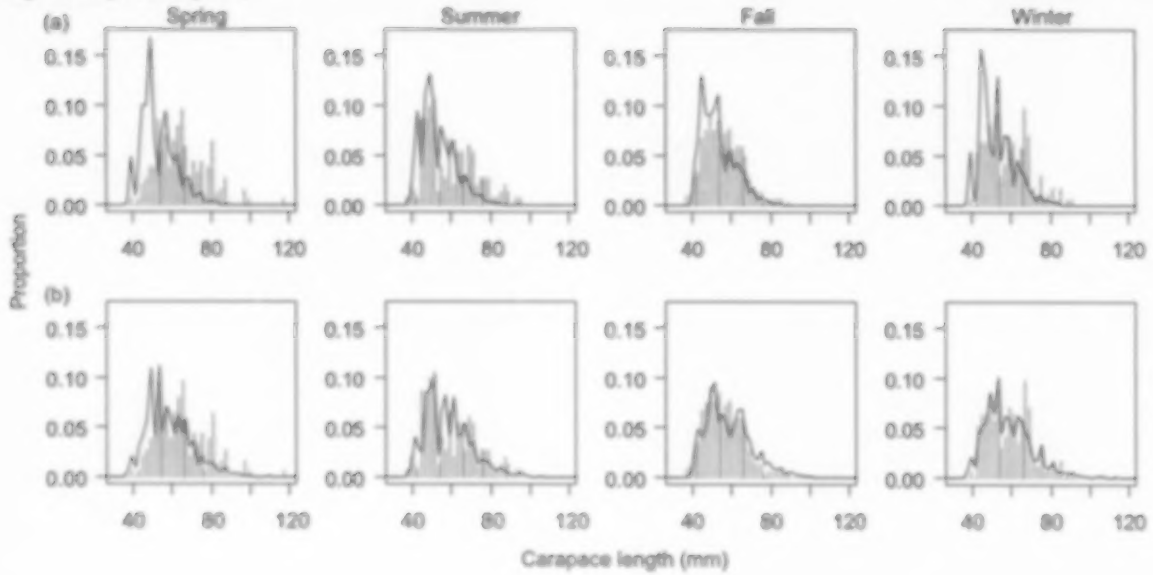
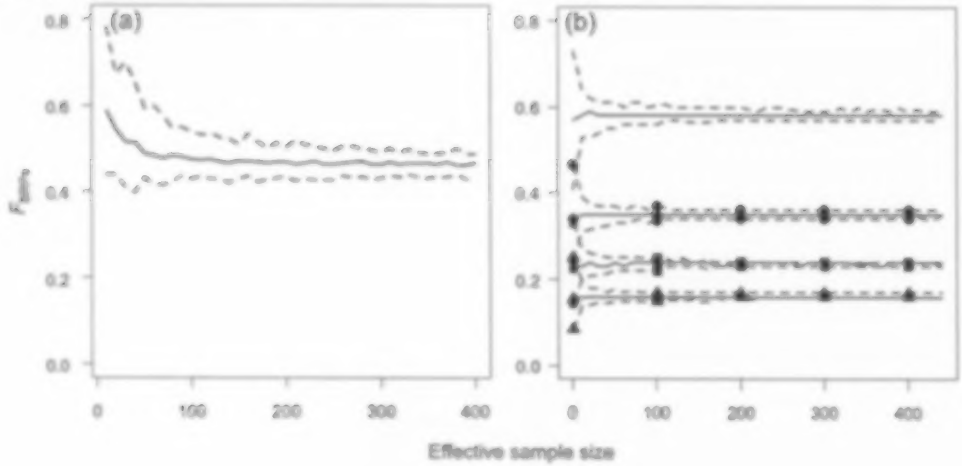


Fig. 3. Evaluating the impacts of the effective sample size on estimates of biological reference points (BRPs): $F_{0.1}$ of the yield-per-recruit analysis (*a*) and $F_{30\%}$ of the eggs-per-recruit analysis (*b*). Solid and dashed lines, respectively, denote the median value and 95% confidence intervals of the distribution of BRPs. For the eggs-per-recruit analysis in panel (*b*), from top to bottom: no symbols, $F_{10\%}$; circles, $F_{20\%}$; squares, $F_{30\%}$; triangles, $F_{40\%}$.



mortality scenario (scenario 3THM) was relatively low (Table 3). Changes in the estimated EPR and BRP values reflected the influence of temperature on the population's egg production through the changes in growth rate, reproductive activity, and mortality. F_{BRPs} values derived from the EPR analysis were more sensitive to a change in temperature and natural mortality compared with $F_{0.1}$ derived from the YPR analysis (Table 3). The composite risks of overexploitation ($P(F_{cur} > F_{BRPs})$) of years 2003–2004 were 0.176, 0.604, 0.951, and 1 for $F_{10\%}$, $F_{20\%}$, $F_{30\%}$, and $F_{40\%}$, respectively. The composite risk of overexploitation decreased with increased temperature when mortality was independent of temperature, particularly for $F_{20\%}$ and $F_{30\%}$ (Fig. 6). Because there was only a slight difference in the F_{BRPs} between scenario CT and scenarios 1TM–3TM, the risks of overexploitation among these scenarios were similar. However, the risk of overexploitation was relatively higher for scenario 3THM (Fig. 6).

Fig. 4. Median of the yield-per-recruit (g) (gray lines) and relative eggs-per-recruit (%) (EPR; black lines) analyses of female spiny lobsters, *Panulirus penicillatus*, as a function of fishing mortality for different simulation scenarios. EPR values of different scenarios are expressed as a percentage of the EPR value when the stock is in a pristine state of scenario CT. The simulation scenarios are indicated as follows: CT, thin solid lines; 1T, thin dashed lines; 2T, thin dotted lines; 3T, thin dashed-dotted lines; 1TM, thick solid lines; 2TM, thick dashed lines; 3TM, thick dotted lines; 3THM, circle-lines (see Table 2 for definitions of the simulation scenarios).

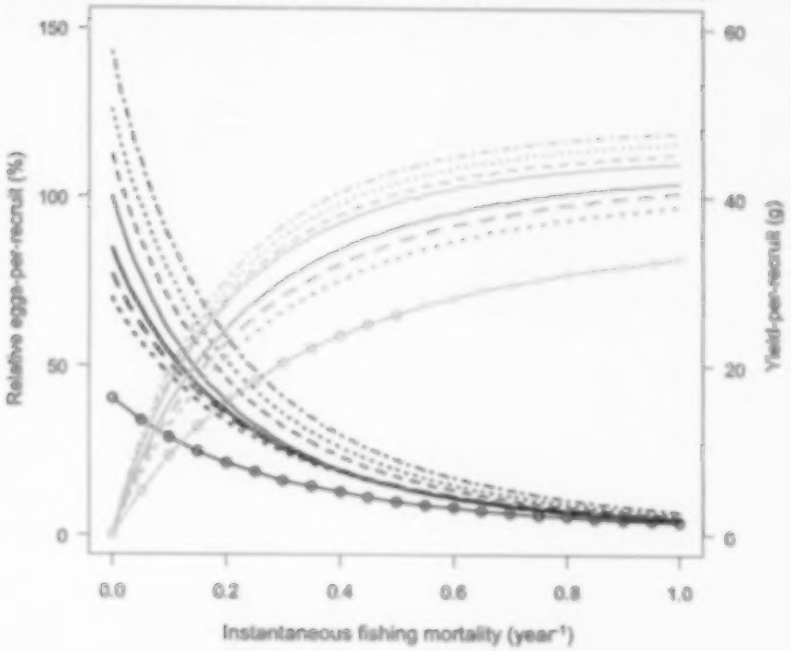


Table 3. Medians of the distribution of biological reference points estimated from the yield-per-recruit ($F_{0.1}$) and eggs-per-recruit analyses ($F_{10\%}$, $F_{20\%}$, $F_{30\%}$, and $F_{40\%}$) for different simulation scenarios.

	Biological reference point (year^{-1})				
Scenario	$F_{0.1}$	$F_{10\%}$	$F_{20\%}$	$F_{30\%}$	$F_{40\%}$
CT	0.46 (4.34%)	0.63 (0.65%)	0.39 (1.18%)	0.27 (0.37%)	0.19 (0%)
1T	0.45 (3.46%)	0.72 (1.04%)	0.45 (1.26%)	0.32 (1.60%)	0.23 (2.10%)
2T	0.47 (3.38%)	0.76 (1.08%)	0.49 (1.29%)	0.35 (1.40%)	0.26 (1.94%)
3T	0.47(3.14%)	0.82 (1.01%)	0.53 (1.18%)	0.39 (1.38%)	0.30 (1.54%)
1TM	0.47 (3.17%)	0.64 (1.08%)	0.38 (1.36%)	0.25 (1.89%)	0.17 (2.09%)
2TM	0.49 (3.95%)	0.65 (1.21%)	0.38 (1.45%)	0.25 (2.08%)	0.16 (2.45%)
3TM	0.49 (3.63%)	0.65 (1.30%)	0.37 (1.55%)	0.23 (2.37%)	0.14 (3.48%)
3THM	0.48 (3.83%)	0.50 (1.63%)	0.22 (2.87%)	0.08 (6.05%)	NA[a]
Scenario 1 of Chang et al. 2009	0.46 (26%)	0.45 (132%)	0.40 (73%)	0.25 (48%)	0.21 (35%)

Note: Numbers in parentheses denote the coefficient of variation of estimates. Estimates of biological reference points for scenarios 1T–3THM are based on the productivity level of scenario CT.

[a]The biological reference points could not be defined.

Evaluation of size regulations

A set of minimum legal sizes (40, 45, 50, and 55 mm CL) was evaluated for their impacts on the YPR and EPR and for their effectiveness in reducing the risk of biological overfishing under the high temperature extra-high mortality scenario (i.e., scenario 3THM of Table 2). For the EPR analysis, increasing the MLS would result in an increase in the EPR value (Table 4). For example, the EPR at $F_{20\%}$ increased by 4% to 32% with increases in the MLS compared with the EPR at $F_{20\%}$ with no MLS (0.22 year^{-1}). Therefore, a higher F_{BRP} would result from implementation of size regulations compared with F_{BRP_0} without MLS. There was also a declining pattern of risk of overexploitation ($P(F_{cur} > F_{BRP_0})$) observed in $F_{10\%}$ and $F_{20\%}$ with an increased MLS except for $F_{30\%}$ and $F_{40\%}$ (Table 5). This implies that the risk of recruitment overfishing of the population can be effectively reduced through an increase of selected MLS (from 40 to 55 by 5 mm interval) for the assumed extra-high mortality environment. However, there was little effect of the MLS on the YPR. The results suggest that increasing

Fig. 5. Boxplots of the estimated biological reference points $F_{10\%}$ (*a*), $F_{20\%}$ (*b*), $F_{30\%}$ (*c*), and $F_{40\%}$ (*d*) of the eggs-per-recruit analysis under eight different scenarios and the current fishing mortality rate (F_{cur}) for female spiny lobsters, *Panulirus penicillatus* (see Table 2 for definitions of the simulation scenarios).

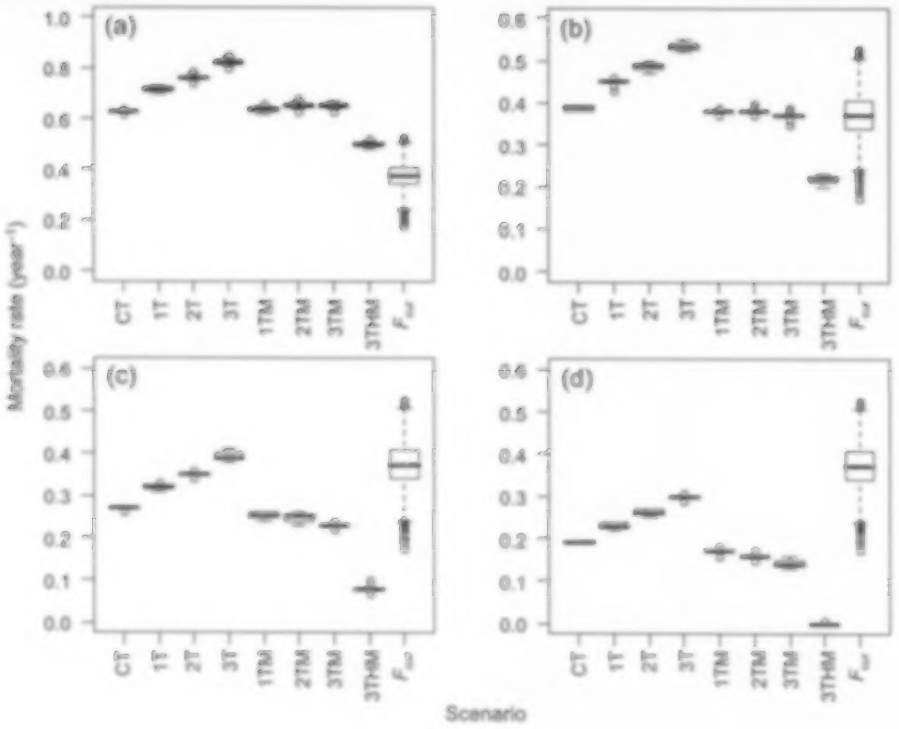
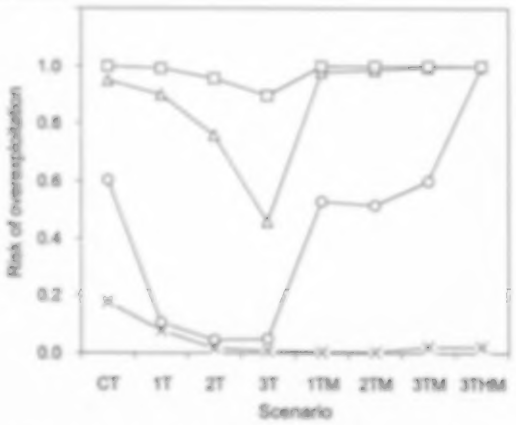


Fig. 6. Estimated risk of overexploitation ($P(F_{cur} > F_{SBPR})$) based on the distribution of current fishing mortality (F_{cur}) and biological reference points (F_{SBPR}: $F_{10\%}$ (crosses), $F_{20\%}$ (circles), $F_{30\%}$ (triangles), and $F_{40\%}$ (squares)) of the eggs-per-recruit analysis under different simulation scenarios for female spiny lobsters, *Panulirus penicillatus*.



the MLS would result in a reduction in the YPR compared with the YPR with no size regulation (Table 4). For example, the YPR at $F_{0.1}$ decreased by 36% to 48% with an increase in the MLS compared with the YPR at $F_{0.1}$ without the size regulation. However, $F_{0.1}$ was not sensitive to an increase in the MLS. Results of scenarios 1–5 suggest that there was a slight decreasing trend in the risk of overexploitation ($P(F_{cur} > F_{0.1})$) with an increased MLS (Table 5). This implies that the MLS management strategy might not be effective in reducing the risk of overfishing of the population.

Discussion

Evaluation of the IBM performance

To evaluate the IBM performance, the predicted size distribution of a pseudocohort was compared with the observed size distribution (combining 2003 and 2004). The results should, however, be carefully interpreted because we used the predicted size composition of a year class with the size composition of the landed catch, which implicitly assumes that the predicted size distribution does not change profoundly over time (i.e., equilibrium assumption). Furthermore, the predicted size distribution compared favorably with the observed distributions at low natural mortality and a low fishing mortality rate, suggesting that the uncertainty of parameters in the IBM (i.e., temporal and spatial errors in natural mortality) might also affect the results. While the observed size distribution did not perfectly correlate with the IBM's prediction, we demonstrated that the developed IBM can broadly capture the growth trajectory based on the published estimates of fishing and natural mortality rates. We subsequently used this individual-based simulation model as the basis of the per-recruit analysis.

Table 4. List of biological reference points (F_{BRPs}) of the yield-per-recruit (YPR) ($F_{0.1}$) and eggs-per-recruit (EPR) analyses ($F_{10\%}$, $F_{20\%}$, and $F_{30\%}$) for different minimum legal regulations under the extra-high mortality scenario (scenario 3THM in Table 2).

	Biological reference point (year^{-1})			
Minimum legal size (mm)	$F_{0.1}$	$F_{10\%}$	$F_{20\%}$	$F_{30\%}$
None	0.45 [38.51]	0.50 [40.57]	0.22 [81.12]	0.08 [121.54]
40	0.48 [25.72] (24.59)	0.50 (42.04)	0.22 (84.23)	0.08 (126.83)
45	0.48 [25.43] (24.46)	0.56 (47.12)	0.24 (88.39)	0.09 (129.01)
50	0.47 [23.58] (23.02)	0.74 (58.22)	0.30 (97.08)	0.11 (133.52)
55	0.47 [20.46] (19.89)	NA[a] (72.07)	0.39 (106.72)	0.14 (137.13)

Note: $F_{40\%}$ is not listed because $F_{40\%}$ could not be defined. Numbers in brackets denote the YPR (g) and EPR (×1000) at a fishing mortality equal to F_{BRPs}. Numbers in parentheses denote the YPR and EPR at a fishing mortality equal to F_{BRPs} with no regulation.

[a]Could not be defined.

Table 5. List of the estimated risks of overexploitation ($P(F_{cur} > F_{BRPs})$) based on the distribution of current fishing mortality (F_{cur}) and biological reference points (F_{BRPs}) of the yield-per-recruit analysis ($F_{0.1}$) and eggs-per-recruit analysis ($F_{10\%}$, $F_{20\%}$, and $F_{30\%}$) for different minimum legal regulations under the extra-high mortality scenario (scenario 3THM in Table 2).

	Risk of overexploitation			
Minimum legal size (mm)	$F_{0.1}$	$F_{10\%}$	$F_{20\%}$	$F_{30\%}$
None	0.07	0.20	1.00	1.00
40	0.03	0.10	1.00	1.00
45	0.03	0.02	0.99	1.00
50	0.05	0.002	0.90	1.00
55	0.05	NA[a]	0.42	1.00

Note: The estimated risks based on $F_{40\%}$ are not listed because $F_{40\%}$ could not be defined.

[a]Could not be defined.

There are a few caveats for the IBM developed in this study that should be noted. Lack of consideration for the reduced number of eggs with repeated spawning within the same season would likely overestimate the total egg production of the population (Chang et al. 2007). Estimation of egg production is independent of males in our model. However, some experimental studies indicated that male sizes, previous copulations, and the balance of expected reproductive return and future mating opportunities may limit the amount of sperm a male transfers to a female, thus limiting egg production (e.g., MacDiarmid and Butler 1999). The schedule of repeated spawnings of *P. penicillatus* in a year may impact on the estimated EPR values. Breeding of *P. penicillatus* occurs practically throughout the year (majority in summer season), but there are insufficient field data to estimate reliably the size-dependent number of spawnings per female in a year. According to the higher incidence of redeveloping gonads occuring in the months of higher temperature, we modeled the individuals to have opportunities to repetitively breed when temperature > 25 °C (Juinio 1987; Plaut 1993; Chang et al. 2007). However, it is unlikely that the reproductive activities correlate with temperature alone. Furthermore, the model does not account for larval connectivity that may buffer or increase the sensitivity of a population to changes in the environment to varying extents (Xue et al. 2008).

Our purpose is not to present a detailed model as some ultimate truth; rather we present a framework for incorporating temperature and other possible environmental variables in simulating the dynamics of the lobster. Traditional population dynamics models, although useful, greatly oversimplify the dynamic processes (e.g., rarely to describe the temperature-dependent discontinuous growth and multiple spawning for spiny lobsters). These models combine many individuals into one group, assuming these individuals will mature, spawn, grow, and die at the same rates over their life span. Although the "average" response produces worthwhile results depending on the questions being asked, individual variability can have a decisive influence on population dynamics (Lowerre-Barbieri et al. 1998). The lobster population exhibiting substantial individual variation might better be analyzed at a lower level of aggregation based on the individual-based approach than on conventional population models. IBMs are widely used in lobster research. For example, IBMs were used for evaluating the performance of stock assessment models (Chen et al. 2005), examining the effects of habitat quality on lobster recruitment (Butler 2003), and exploring the connectivity of lobster populations (Xue et al. 2008).

This study shows that changes in thermal regimes can affect estimates of YPR and EPR values, BRPs, and risk assessment. An increasing temperature can increase the stock productivity through shortening the intermolt period (faster growth), together with changes in the spawning season, spawning times, and incubation duration when there is no effect of temperature change on mortality. Therefore, warming water can reduce the risk of overexploitation in the long term (scenarios CT–3T) under a constant harvest rate management policy. The stock productivity is relative stable because of the offset between increased growth rate, reproductive activity and mortality when mortality is related to the temperature changes. However, the stock productivity is likely to be reduced, leading to a higher risk of overexploitation, if higher temperatures introduce extra-high mortality (scenario 3THM). The most immediate implication for the management of fisheries is that current BRPs developed for precautionary management might not be suitable in the long run if climate conditions greatly change. This is partic-

ularly important for managing a declining population and should be of concern for stock recovery plans (MacCall 2002; Cook and Heath 2005; Köster et al. 2009).

We evaluated the relative importance of various biological processes (i.e., temperature-dependent growth, reproduction, and mortality) that determine the stock productivity based on the individual-based per-recruit analysis. This suggests that natural mortality is most important. Similar conclusions on the effects of natural mortality can be found in estimating the uncertainty of BRPs (Chen and Wilson 2002; Jiao et al. 2005; Chang et al. 2009). Natural mortality is one of the essential life history parameters in the Beverton and Holt (1957) theory of fishing. In this study, we suggest that the generic mortality ($year^{-1}$) increased with temperature based on Pauly's (1980) empirical function given the K–temperature proportional relationship derived from various lobsters. K depends on the activity and metabolism of the animal and may be influenced by temperature; therefore, temperature affects the food intake of predators and hence predation mortality in a similar way (Pauly 1980). Furthermore, temperature may also affect mortality directly by increasing physiological mortality caused solely by disease or old age (Pauly 1980; Brown et al. 2004). Previous studies suggested that warming temperatures might affect mortality of the lobster. Dove et al. (2004) indicated that there was a strong correlation between a disease (excretory calcinosis) and high temperatures in Long Island Sound bottom waters, which resulted in high natural mortality of American lobster during 1999–2002. It was shown that temperature could negatively affect the immune system of crustaceans (Le Moullac and Haffner 2000). Furthermore, increasing atmospheric concentrations of carbon dioxide (CO_2) also altered the seawater chemistry (e.g., ocean acidification). Kurihara et al. (2008) suggested that predicted future seawater CO_2 conditions would potentially reduce marine shrimp (*Palaemon pacificus*) and possibly other crustacean populations through negatively affecting mortality, growth, and reproduction. It is important to understand biological mechanisms through which climate changes affect population dynamics of organisms to quantify the magnitudes of potential changes and improve the assessment framework. The framework developed in this study provides a practical approach to quantify possible consequences of changes in temperature and other key environmental variables on lobster population dynamics.

The individual-based per-recruit analysis suggested that the current fishing mortality (0.37 in 2003–2004) was lower than $F_{0.1}$ (0.46), $F_{10\%}$ (0.63), and $F_{20\%}$ (0.39); but higher than $F_{30\%}$ (0.27) and $F_{40\%}$ (0.19) estimated based on the current environment (i.e., scenario CT). A comparison of the median values of the F_{BRP} distributions derived from scenario CT in the IBM and the conventional per-recruit model (age-structured per-recruit model; see scenario 1 in Chang et al. 2009) suggests that the two models yield similar $F_{0.1}$, $F_{20\%}$, $F_{30\%}$, and $F_{40\%}$, but slightly different $F_{10\%}$. The difference in $F_{10\%}$ between the two methods might result from the $F_{10\%}$ estimates in Chang et al. (2009) having a large proportion of poorly defined runs when the input parameters' uncertainty was incorporated in the EPR analysis. Comparison of F_{BRP} estimates also suggests that the age-structured per-recruit model, which uses the VBGF for growth modelling, yields reliable estimations of BRPs. This implies that the VBGF, which is commonly used in conventional stock assessment models (Smith and Addison 2003), is still applicable for crustacean species. Similar results were also observed for the blue crab (*Callinectes sapidus*) in Chesapeake Bay (Bunnell and Miller 2005).

Although the IBM can mimic the life history processes of the lobster (e.g., temperature-dependent molting process), the uncertainty of F_{BRPs} of the individual-based per-recruit analysis tends to be smaller than that for the age-structured per-recruit analysis. Such a difference in F_{BRPs} may result from a lack of considering process errors (e.g., uncertainty of the natural mortality rate) in the individual-based per-recruit model. Because of the long computational time of the present model, the uncertainty associated with the input parameters was not included in the current simulation. However, the developed model is capable of incorporating these uncertainties. We suggest that the process error be considered in estimating BRPs using the IBM.

$F_{0.1}$ is an arbitrary value that has no optimal properties or theoretical basis; however, it has gained acceptance as a management target in fisheries (Deriso 1987; Hilborn and Walters 1992). The effectiveness and appropriateness of using $F_{20\%}$ and $F_{40\%}$ or $F_{0.1}$ as proposed for F_{BRPs} for assessing the lobster stock in Taitung waters were evaluated and questioned (Chang et al. 2009). The key question lies in their ad hoc nature and uncertainty in the stock–recruitment relationship for the spiny lobster. In this study, all scenarios show that YPR and EPR values are sensitive to changes in temperature. However, impacts of temperature on $F_{0.1}$ were much smaller compared with its impacts on F_{BRPs} (e.g., $F_{20\%}$) estimated from the EPR analysis. This study suggests that $F_{0.1}$ may be robust to the uncertainty in environmental variables (e.g., temperature) and is a good candidate of target BRPs.

For the defined high temperature extra-high mortality scenario, an increase in the MLS resulted in increases in EPR and F_{BRPs}. However, increasing the MLS did not result in a large change in YPR or $F_{0.1}$. Thus, the goals of reducing the risks of both growth and recruitment overfishing do not conflict. The risk of biological overfishing in an unfavorable environment can be reduced by adapting a minimum size regulation of 55 mm CL, which is approximately the 50% physiological size-at-maturity of *P. penicillatus* (Chang et al. 2007). A major constraint for the EPR analysis is that an increase in egg production did not necessarily result in an increase in recruitment at a later date. The effects of changes in egg production on subsequent recruitment vary, depending on the functional form of the stock–recruitment relationship. Recruitment is likely to decline and ultimately fail if the stock size is reduced to a very low level and the environment is not favorable for the survival of animals in the early life stages.

Many approaches can be used to reduce fishing efforts in a crustacean fishery: (*i*) size regulations (minimum and maximum legal sizes); (*ii*) protection of berried and reproductively active females; (*iii*) seasonal closures; (*iv*) protection of softshell lobster; and (or) (*v*) area closures (see tables 11.1 and 12.1 of Phillips and Melville-Smith (2006)). The minimum legal size is commonly chosen as a tool for managing crustacean stocks (Gendron 2005). However, the maximum

legal size is also suggested to be effective in conserving a stock's reproductive potential because larger females are highly fecund; produce large, high-quality eggs; and are multiparous compared with males (Ouellet and Plante 2004). Environmental conditions can also affect catches through influencing catchability. For example, increasing water temperature positively affects the catchability of rock lobsters, *P. cygnus* (Morgan 1974; de Lestang et al. 2009). Thus a long-term increase in water temperature may increase fishing efficiency, and this is particularly important for fisheries managed with effort controls.

This study suggests that the conventional age-structured or size-structured per-recruit model can provide reliable estimates of BRPs even though it does not mimic the nature of the stepwise growth of the lobster. This study demonstrates that thermal changes can affect stock productivity and estimates of BRPs, and there is a high risk of overexploitation in the long term if high temperature introduces extra-high mortality. In an unfavorable environment, increasing the minimum legal size is recommended as a potentially optimal measure to reduce the risk of overexploitation in the long run. This study highlights the importance of incorporating environmental variables and subsequent uncertainties in fisheries assessments.

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Appendix A

Table A1. List of parameters (L_∞ and K) of von Bertalanffy growth function for various lobster species in family Palinuridae.

Species	Region	Sex	SST (°C)	L_∞ (mm)	K (year^{-1})	Reference
Jasus edwardsii	Fiordland coast, southwest coast of New Zealand	M	14.5	202	0.13	Annala and Bycroft 1988
		F	14.5	165	0.04	
Jasus lalandii	Southern South Africa	M	19.5	111.2	0.09	Groeneveld 1997
		F	19.5	96.1	0.13	
Palinurus elephas	Central–western Sardinia (central–western Mediterranean Sea)	M	18.2	167.9	0.13	Follesa et al. 2007
		F	18.2	120.2	0.21	
Panulirus cygnus	Seven Mile Beach, Western Australia	M	21.3	158.8	0.17	Sheehy et al. 1998
		F	21.3	125	0.19	
Palinurus delagoae	Eastern coast of South Africa	C	24.2	139.25	0.07	Groeneveld 2000
Panulirus argus	Gulf of Batabanó, southwestern Cuba	M	28.1	250.3	0.27	Phillips et al. 1992
		F	28.1	170.9	0.39	
Panulirus marginatus	Necker Island, Hawaiian Archipelago	M	25.4	136.1	0.27	O'Malley 2009
		F	25.4	122.8	0.35	
Panulirus homarus	Durban, eastern coast of South Africa	M	23.2	120	0.18	Areffano 1989
		F	23.2	94.2	0.34	
Panulirus ornatus	Torres Strait, Australia	M	27.9	162.35	0.45	Pitcher et al. 1992
		F	27.9	144.23	0.58	
Panulirus longipes	Isle of Pines, New Caledonia	M	24.5	131	0.39	Coutures and Chauvet 2001
		F	24.5	113	0.44	
Panulirus versicolor	Southern section of Great Barrier Reef, Australia	C	23.2	144.7	0.27	Frisch 2007
Panulirus penicillatus	Enewetak Atoll, Marshall Islands	M	28.3	146.5	0.21	Ebert and Ford 1986
		F	28.3	96.5	0.58	
	Solomon Islands	M	29.0	143.9	0.29	Prescott 1988
		F	29.0	113.4	0.50	
	Tongatapu, Kingdom of Tonga	M	25.7	179	0.27	Munro1988
		F	25.7	128	0.32	
	Isle of Pines, New Caledonia	M	24.5	176	0.26	Coutures and Chauvet 2001
		F	24.5	143	0.32	
	Galápagos Marine Reserve	M	22.6	169.1	0.20	Hearn and Murillo 2008
		F	22.6	123.4	0.26	
	Eastern coast off Taiwan	M	26.4	135.6	0.19	Derived from data of Chen 2005
		F	26.4	105.6	0.22	

Note: Data for SST (annual mean sea-surface temperature) is taken from Physical Sciences Division of NOAA–ESRL: http://www.esrl.noaa.gov/psd/data/. M, males; F, females; C, sex combined.

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Sources of variability and comparability between salmonid stomach contents and isotopic analyses: study design lessons and recommendations

Mark R. Vinson and Phaedra Budy

Abstract: We compared sources of variability and cost in paired stomach content and stable isotope samples from three salmonid species collected in September 2001–2005 and describe the relative information provided by each method in terms of measuring diet overlap and food web study design. Based on diet analyses, diet overlap among brown trout, rainbow trout, and mountain whitefish was high, and we observed little variation in diets among years. In contrast, for sample sizes $n \geq 25$, 95% confidence interval (CI) around mean $\delta^{15}N$ and $\delta^{13}C$ for the three target species did not overlap, and species, year, and fish size effects were significantly different, implying that these species likely consumed similar prey but in different proportions. Stable isotope processing costs were US$12 per sample, while stomach content analysis costs averaged US$25.49 ± $2.91 (95% CI) and ranged from US$1.50 for an empty stomach to US$291.50 for a sample with 2330 items. Precision in both $\delta^{15}N$ and $\delta^{13}C$ and mean diet overlap values based on stomach contents increased considerably up to a sample size of $n = 10$ and plateaued around $n = 25$, with little further increase in precision.

Résumé : Nous avons comparé les sources de variabilité et les coûts dans des échantillons appariés de contenus stomacaux et d'isotopes stables récoltés en septembre 2001–2005 chez trois espèces de salmonidés; nous décrivons l'information relative apportée par chaque méthode en ce qui a trait au chevauchement des régimes alimentaires et au plan d'étude des réseaux trophiques. D'après les analyses des régimes alimentaires, le chevauchement est élevé entre la truite brune, la truite arc-en-ciel et le ménomini de montagnes et il y a peu de variation de régime entre les années. En revanche, pour des tailles d'échantillon de $n \geq 25$, les intervalles de confiance (IC) à 95 % autour des valeurs moyennes de $\delta^{15}N$ et de $\delta^{13}C$ pour les trois espèces ciblées ne se chevauchent pas et les effets de l'espèce, de l'année et de la taille du poisson sont significativement différents, ce qui implique que ces espèces consument vraisemblablement des proies semblables, mais dans des proportions différentes. Le coût de traitement des analyses d'isotopes stables est de 12 $US par échantillon, alors qu'une analyse de contenu stomacal coûte en moyenne 25,49 ± 2,91 $US (IC à 95 %) et varie de 1,50 $US pour un tube digestif vide à 291,50 $US pour un échantillon contenant 2330 proies. La précision du chevauchement moyen des régimes alimentaires basé sur les valeurs de $\delta^{15}N$ et de $\delta^{13}C$ et sur les contenus stomacaux augmente considérablement jusqu'à une taille d'échantillon de $n = 10$ et atteint un plateau vers $n = 25$ avec peu d'augmentation de précision par la suite.

[Traduit par la Rédaction]

Introduction

Collection and analysis of stomach content and stable isotope data both provide a means to describe trophic interactions among species or feeding guilds. This information has been used to quantify resource overlap between species (e.g., Schoener 1970; Vander Zanden et al. 1997), assess effects of fish management decisions (e.g., Harvey and Kitchell 2000), and evaluate the potential impacts of invasive species on natural food webs (e.g., Vinson and Baker 2008). Our ability to detect biologically relevant, statistical differences among trophic levels or between species diets is determined by the variability in the measurement around mean values and the number of replicate samples, as well as the complexity of the food web. Primary sources of variation in both stomach contents and stable isotopes are ontogenetic changes in diet (Genner et al. 2003), seasonal diet shifts (Vizzini and Mazzola 2003; Perga and Gerdeaux 2005), and variance in prey selection among individuals (Beaudoin et al. 1999).

Historically, the characterization of diets and trophic linkages among fish species relied primarily on evaluation of stomach contents (Hyslop 1980; Bowen 1983). The collection and analysis of stomach content data, however, has a

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M.R. Vinson.[1] United States Geological Survey, Great Lakes Science Center, Lake Superior Biological Station, 2800 Lake Shore Drive, Ashland, WI 54806, USA.
P. Budy. United States Geological Survey, Utah Cooperative Fish and Wildlife Unit, Department of Watershed Sciences, Utah State University, Logan, UT 84322-5210, USA.

[1]Corresponding author (e-mail: mvinson@usgs.gov).

number of drawbacks; it can be detrimental to the study animal; the material can be laborious to identify; and stomach contents can be highly variable based on variation in digestion rates, feeding habits, seasonal or diel collection times, fish size, and individual dietary whims (Bowen 1983). Traditional stomach content measurements include counts, frequency of occurrence, and volume or weight of individual prey items (Hyslop 1980). While a number of indices incorporating one of more of these measurements have been developed to describe the diets of individual species and to assess trophic position and diet overlap among species (Cailliet and Barry 1979; Hyslop 1980; Cortés 1997), there has been a lack of consistency in the application of statistical tests to analyze these metrics, which has hindered comparative studies (reviewed by Cortés 1997). The reporting of measures of variance associated with stomach content data appears particularly problematic. In a review of more than 200 diet studies, Ferry and Cailliet (1996) reported that none of the studies they reviewed provided any estimates of precision in describing diet composition.

In the last 30 years, stomach content analyses have been complemented or replaced by analysis of stable isotope ratios, principally nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$), for the determination of the relative trophic position of organisms or species (or "who eats whom"; Peterson and Fry 1987). In comparison with stomach content data, stable isotope data can provide longer-term dietary inferences, reduced labor in sample collection and preparation, and collection methods are typically nonlethal. Stable isotopes integrate dietary patterns over weeks, months, or even years (Hesslein et al. 1993) instead of hours or days for stomach contents. The integration of the dietary regime over time may reduce variation among individuals as compared with stomach contents and potentially provide more direct evidence for which diet items are most important in terms of actually being assimilated into tissue over time (Vinson and Baker 2008). Given the potential to improve our ability to infer trophic differences among species, this trait (time integration and assimilation) makes stable isotopes especially desirable in ecological studies (Vander Zanden et al. 1997; Harvey and Kitchell 2000; Kelly et al. 2004). In contrast with stomach content measures, which are often categorical or proportion data, stable isotope data are continuous numeric data facilitating the statistical detection of biologically relevant patterns (Hobson 1993).

For stomach contents, the occurrence of empty stomachs and our ability to identify partially digested prey can also increase variation in diet measurements. In a review of stomach content data from 254 fish species, Arrington et al. (2002) reported the number of empty stomachs averaged 16% and varied from 0% to 79% among individual species. The occurrence of unidentifiable prey in stomachs appears similar, but is strongly influenced by prey type, with planktivores and invertivores having more unidentifiable material in their stomachs than piscivores.

In addition to these primary sources of variation in both stomach contents and stable isotopes, additional error may occur for stable isotopes when associated with sample collection, preparation, or analytical measurement, and differences among tissue types within individual specimens have been documented. Variation in stable isotope values due to sample collection and preparation errors is likely minor (Jacob et al. 2005), as are analytical errors associated with isotope ratio mass spectrometry (Jardine and Cunjak 2005). In general, variation among fish tissues appears slight: McCarthy and Waldron (2000) — brown trout (*Salmo trutta*); Shannon et al. (2001) — humpback chub (*Gila cypha*); Finlay et al. (2002) — rainbow trout (*Oncorhynchus mykiss*); Jardine et al. (2005) — Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*); Sanderson et al. (2009) — Chinook salmon (*Oncorhynchus tshawytscha*). However, Suzuki et al. (2005) and Perga and Gerdeaux (2005) both measured differences among tissue types, and the difference or rate of isotopic change among tissues in response to changes in diet appears to be directly related to tissue growth (Hesslein et al. 1993). Thus, changes in isotopic composition in slow-growing fish or slow-growing tissue could take months or years. Overall, variation in stomach contents and stable isotopes appear most strongly influenced by ontogenetic changes, seasonal differences, and individual variation among individuals. Variation in diets due to ontogenetic changes may be reduced by stratifying analyses by fish size. Sampling within a discrete sampling period or stratifying analyses by season can reduce seasonal differences.

This study analyzed paired stomach content and isotopic samples from the same individuals to evaluate the effects of sample size on the ability to resolve dietary overlap and differences in isotopic signatures and subsequently to compare inferences about trophic structure made from each data type alone (stomach, isotopic data) vs. both data types together. We examined stomach contents and muscle isotopic signatures in three species of salmonids that function as top predators (brown trout, rainbow trout, mountain whitefish (*Prosopium williamsoni*)) in a stream ecosystem. Specifically, we investigated (*i*) variation in the stomach contents and $\delta^{13}C/\delta^{15}N$ values among individuals, among species, within years, and among years; (*ii*) the influence of sample size on variation and precision of mean dietary overlap and isotopic composition; and (*iii*) precision gained vs. monetary cost of processing additional diet or isotope data.

The overall goal of this study was to answer two very broad and important questions in applied ecology. (*i*) As compared with stomach content samples, what information do stable isotope samples provide and how many do you need? Within this context, (*ii*) what information do stomach content samples provide relative to stable isotope samples? Specifically, we capitalized on the opportunity to use a large data set of paired isotope samples and stomach content data to evaluate sources of variability and to aide in designing food web studies. For three salmonid species, we report (*i*) the variation in stomach contents and $\delta^{13}C$ and $\delta^{15}N$ values among individuals within and among years; (*ii*) variation in $\delta^{13}C$ and $\delta^{15}N$ values within individual fish and between samples collected from muscle tissue versus adipose fins; (*iii*) the influence of sample size on the magnitude of variation around mean diet overlap and $\delta^{13}C$ and $\delta^{15}N$ values among the three species attributed to interindividual differences; and (*iv*) we consider the implications of this variation for inferring trophic position from stable isotopes, a common goal of food web studies. We finish with a comparison of costs associated with processing stomach samples relative

to stable isotope samples, and a cost–benefit analysis of the precision gained with increasing sample size relative to the increasing cost of taking and analyzing more isotope samples.

Materials and methods

Study area

Fish and invertebrate samples were collected from the Green River downstream from Flaming Gorge Dam in northeastern Utah (40°54′N, 109°25′W). Vinson (2001) described the hydrology and biology of this reach in detail. The river can be characterized as clear and cold (2–14 °C annual temperature range), with deep runs (2–4 m) and pools (5–12 m), large cobble and boulder substrates, and abundant growth of aquatic plants that includes moss (*Amblystegium riparium*), *Cladophora glomerata*, *Chara* sp., *Elodea canadensis*, *Potamogeton crispus*, *Potamogeton pectinatus*, *Ranunculus* sp., and *Spirogyra* sp. Aquatic invertebrate assemblages are characteristic of a hypolimnetic release dam-influenced river and are dominated by Chironomidae, Simuliidae, Amphipoda, and *Baetis* mayflies (Vinson 2001). Fish populations are dominated by brown trout and rainbow trout, with fewer numbers of mountain whitefish and mottled sculpin (*Cottus bairdii*) present. Harju (2007) evaluated the potential influence of New Zealand mud snails (*Potamopyrgus antipodarum*) on the food web of this river reach using results from an isotope mixing model (Phillips and Gregg 2003) and a bioenergetics model and provides additional stable isotope data on other members of the food web not described here.

Field collections

Salmonid species and mottled sculpin were collected in proportion to their natural abundances by Utah Division of Wildlife personnel using electrofishing techniques for 2001–2005. Samples were collected each September from two ~10 km long stream reaches — just downstream from Flaming Gorge Dam and downstream from Little Hole — on two consecutive nights. All fish were collected at night between 20:00 and 23:00. Stomach contents were collected from 50 fish randomly selected at each location each year. Once collected, fish were anesthetized with tricaine methanesulfonate (MS-222), identified, measured to the nearest millimetre, weighed to the nearest 0.1 kg, and had their stomach contents removed by pulsed gastric irrigation (Light et al. 1983). Stomach contents were preserved in the field in 95% ethanol and returned to the laboratory. For stable isotope analyses, a single white muscle tissue sample was collected from below the dorsal fin with a 5 mm diameter dermal biopsy punch (Miltex Instrument Company, Bethpage, New York). The muscle plug was placed on ice for several hours and then frozen. The fish were then placed in a pen in the river to recover and then released. Aquatic invertebrates were collected for $\delta^{13}C$ and $\delta^{15}N$ analysis each September between 2001 and 2005 from the Green River in the same locations as the fish were collected. Invertebrates were collected qualitatively with kick nets, kept on ice in the field, and then frozen until processed.

Laboratory methods

Stomach contents

Stomach content samples were identified in the laboratory under a dissection microscope, generally to genus or species, and counted. Partially digested contents were identified and enumerated as conservatively as possible. For example, the presence of two invertebrate eyes was counted as one individual and unidentifiable fish bones were recorded as a single undetermined fish. Stomach content data were summarized into 10 major categories: Amphipoda (mostly *Hyalella azteca* and some *Gammarus lacustris*); Coleoptera (mostly Elmidae, *Optioservus*); Diptera (mostly Chironomidae and Simuliidae); Ephemeroptera (mostly *Baetis tricaudatus*); Mollusca (mostly Gastropoda, *Potamopyrgus antipodarum*); and others (Plecoptera (*Hesperoperla pacifica*), Trichoptera (mostly Hydroptilidae, *Hesperophylax*, and *Rhyacophila*), and Annelida (mostly Oligochaeta)), terrestrial invertebrates, deer mice (*Peromyscus maniculatus*), mottled sculpin, juvenile salmonids, and salmonid eggs.

Stable isotopes

Stable isotope samples were processed within a few weeks of collection. Invertebrates were identified to genus or species prior to processing. Individual invertebrates were cleaned of debris by rinsing them with deionized water. Snails were removed from their shells. All stable isotope material was dried for at least 48 h at 65 °C (Midwood and Boutton 1998), ground to a fine powder with a mortar and pestle, and packed in 8 mm × 5 mm tin capsules. Two milligrams of plants, invertebrates, and fish were used for isotopic analysis. When possible, one invertebrate was used to meet the mass requirement; if this was not possible, multiple organisms of the same taxa were pooled to meet the minimum requirement. Individual fish tissue samples were analyzed separately. $\delta^{13}C$ and $\delta^{15}N$ content was determined using a Europa Hydra 20/20 continuous flow isotope ratio mass spectrometer at the University of California–Davis Stable Isotope Facility (Davis, California, USA). Stable isotope contents are reported in del (δ) units, in parts per thousand (‰) relative to the reference standards for PeeDee belemnite for ^{13}C and nitrogen gas in ambient air for ^{15}N, calculated as follows:

$$\delta^{13}C \text{ or } \delta^{15}N = [(\text{Rsample}/\text{Rstandard}) - 1] \times 1000$$

where $R = {}^{13}CO_2/CO_2$ for $\delta^{13}C$ or $R = {}^{15}N/N_2$ for $\delta^{15}N$.

Data analysis

To reduce variation associated with ontogenetic changes in diet and best address our study design-based objectives, we included only salmonids > 250 mm or a size threshold that corresponds to fish age-2 or older. Previous work on this system demonstrated that trout smaller than 250 mm are invertivores, and larger fish are omnivores and piscivores (M.R. Vinson, unpublished data). Our analyses were based on samples collected from 139 brown trout, 33 mountain whitefish, and 111 rainbow trout. The number of salmonids from which paired stomach and isotope samples were collected from each year was 22 in 2001, 72 in 2002, 66 in 2003, 61 in 2004, and 62 in 2005. Brown trout mean total length was 415 mm and mean weight was 0.8 kg (range

250–673 mm, 0.2–3.0 kg). Mountain whitefish mean total length was 451 mm and mean weight was 1.2 kg (range 328–516 mm, 0.5–1.7 kg). Rainbow trout mean total length was 362 mm and mean weight was 0.5 kg (range 250–465 mm, 0.2–1.3 kg). For fish > 250 mm, length and weight did not vary substantially among years for any species. In 2005, three replicate muscle plugs and an adipose fin tissue sample were collected from each of five individual fish of each species. Stable isotope data were also collected from 26 mottled sculpin (27–105 mm), included only for trophic comparisons in the food web analysis described below.

Stomach contents

Nonmetric multidimensional scaling (MDS) was used to examine differences in stomach contents among salmonid species (Primer-E, ver. 6.1.12, Primer-E Ltd., Plymouth, UK). Count data were standardized to the maximum values, so samples were expressed as the percent composition of each prey category following Clarke and Gorley (2006). We used ordinations based on Sorenson/Bray–Curtis similarity distance measurements to provide graphical representations of assemblage patterns. In two-dimensional ordination, samples that group close to one another indicate similar assemblages, whereas samples far apart indicate relatively dissimilar assemblages. Significance of a priori groupings, species and years, were tested with a nested analysis of similarities (ANOSIM) using Primer ver. 6, where species was the main factor being tested, and year was nested within species. We calculated similarity percentages (SIMPER) in Primer to determine if a particular diet category varied widely among salmonids. In this test, the statistic R is a measure of effect size, where $R = 1$ indicates that samples within a group are more similar to each other than members from other groups, and $R = 0$ indicates that within-group similarity is equal to among-group similarity; an R near 1 indicates strong grouping, whereas an R near 0 indicates weak grouping.

We used Schoener's overlap index (Schoener 1970) to evaluate the effect of sample size on precision and mean diet overlap among species. Schoener's index was calculated as

$$\alpha = 1 - 0.5\sum_{i}^{n} p_{x,i} - p_{y,i}$$

where α is the overlap index, $p_{x,i}$ is the percentage of food type i eaten by species x, $p_{y,i}$ is the percentage of food type i eaten by species y, and n is the total number of prey item categories. Values range from 0 (no overlap) to 1 (complete overlap), and overlap is generally considered to be biologically significant when the value exceeds 0.60 (Zaret and Rand 1971). Effect of sample size on diet overlap values was evaluated by randomly selecting fish in sample size bins ranging from $n = 2$ to $n = 30$ and calculating index values based on these fish, repeated 1000 times for each sample size bin with replacement. We then calculated the mean and 95% confidence interval (CI) for each set of random draws.

Stable isotopes

To verify there were no significant intraindividual variation in isotopic signatures, we compared variation in isotopic composition among repeated samples taken within an individual fish (muscle, $n = 3$ for each sample) and the difference and variation between isotopic composition of muscle tissue samples versus tissue samples collected from the adipose fin ($n = 5$ for each tissue type) for each fish species. We then compared mean $\delta^{13}C$ and $\delta^{15}N$ between adipose and mean muscle tissue using pairwise two-sample t tests assuming unequal variance, with statistical significance based on an a priori $\alpha = 0.05$. Among replicated muscle tissue samples ($n = 3$ per fish), we evaluated the average difference in mean $\delta^{13}C$ and $\delta^{15}N$ values among fish and used nonoverlapping 95% CI as an indication of likely significant biological difference (Cumming 2009).

Isotopic variation among individuals and precision versus sample size

We evaluated variation in mean isotopic composition with a general linear model fit to both $\delta^{13}C$ and $\delta^{15}N$ as response variables and both categorical (species and year) and continuous (fish length) predictor variables, including interactions among variables (SAS Institute Inc. 2005). Because of heterogeneous variances among the species, the best model estimated a separate residual variance for each species. Fish length was mean-centered for each species prior to analysis, and we screened all possible interactions involving fish length to determine which were supported by data patterns for both $\delta^{13}C$ and $\delta^{15}N$, using Akaike's information criterion (AIC) to select the most parsimonious model (Burnham and Anderson 1998). Data analyses were obtained using the MIXED procedure in SAS/STAT software (version 9.2) in the SAS System for Windows. AIC values for comparing models that differed in fixed-effects specifications were obtained using the maximum likelihood method.

To evaluate the effect of sample size on the precision with which mean isotopic composition was estimated, we fit an analysis of variance of a two-way factorial in a completely randomized design to both $\delta^{13}C$ and $\delta^{15}N$ as response variables with species, year, and their interaction as categorical predictor variables, specifying heterogeneous variances for species (i.e., a separate residual variance was estimated for each species). The residuals from these models were then used in a bootstrapping exercise to assess the relationship between sample size and precision. Using this approach, the potentially confounding effect of year is factored out, and the analysis focused on the effects of sample size given the effects of variation in fish size on statistical and biological inference. Our evaluations were based on 1000 bootstrap samples of the data for each sample size (increments of 1 for $n = 2$–10, increments of 5 for $n = 10$–50). We set α for confidence limits at 0.05 (95% CI) and applied the bootstrap exercise to each species for both $\delta^{13}C$ and $\delta^{15}N$. Data analyses were obtained using the MIXED procedure in SAS/STAT software (version 9.2), and the %JACKBOOT macro, available from the SAS Web site (http://support.sas.com/kb/24/982.html, accessed 26 June 2010).

Food web and trophic overlap

The effect of sample size differences on the overall depiction of the food web and the degree of diet overlap among species or trophic groups was inferred by the degree of over-

lap in 95% CIs around mean values for different sample sizes. Diets and trophic positions were considered different when 95% CIs did not overlap (Cumming 2009). For lower trophic levels (sculpin, $n = 26$; salmonid eggs, $n = 13$; invertebrate predators, $n = 65$; detritivores, $n = 102$; and herbivores $n = 65$), the mean was determined from a randomly selected set of 25 samples from each lower trophic level (13 for salmonid eggs). This mean was then held constant for a sample size of 25 (as the purpose here is to show the effect of increasing precision), and 95% CIs were calculated based on the mean standard deviation of 100 random drawn samples with replacement for sample sizes of $n = 3$, 5, 10, and 25. Mean and 95% CI for brown trout, rainbow trout, and mountain whitefish were based on the bootstrap analysis described above. Invertebrate species were grouped into predators (principally Diptera (Tabanidae, Muscidae), Odonota (Coenagrionidae), Trichoptera (Rhyacophilidae, *Rhyacophila*)), detritivores (Amphipoda (*Gammarus lacustris* and *Hyalella azteca*), Diptera (Orthocladiinae, *Simulium*), Ephemeroptera (*Baetis tricaudatus*)), and herbivores (Coleoptera (*Optioservus*), Ephemeroptera (*Ephemerella*), Gastropoda (*Potamopyrgus antipodarum* and *Physella*)) based on dietary information in Thorp and Covich (1991) and Merritt et al. (2008).

Costs

Finally, we compared the costs of processing stomach content and stable isotope samples and evaluated the relative benefit of preparing and analyzing more isotope samples in terms of cost versus the increase in precision gained by adding additional samples. Costs for processing stomach content samples were based on the time required to process each sample. A labor cost of $10 per hour (all currency in US$) was used for sorting these samples into gross taxonomic categories (essentially invertebrate orders), $20 per hour for enumerating and identifying the individuals to genus or species, and $15 per hour for entering these data into a computer program. We estimated the cost for stable isotope samples at $12 per sample based on an analysis cost of $8 per sample for $\delta^{13}C$ and $\delta^{15}N$, the price we paid for these analyses over the 5-year study, a laboratory preparation cost of $3 per sample, and an estimated material supply cost of $1 per sample. Data are provided by the stable isotope analysis lab in electronic format, so no data entry cost was included for isotopes. On average, about 0.25 h of technician time is required per sample to dry, crush, and place the material into tin capsules. Supplies include disposable items such as biopsy punches and tin capsules as well as capital items such as a mortar and pestle, an electronic balance, and a drying oven. We did not include the cost for collecting fish in the field, as these costs will vary dramatically across different species and systems, and they are similar whether you are collecting stomach samples or stable isotope samples from these fish. We compare the mean cost for processing stomach sample contents to the fixed $12 per sample we estimated for determining stable isotope values. For stable isotopes, we then evaluate the relationship between isotopic value precision (95% CI), costs, and sample size using the data from the previous analysis. Minor differences or error in cost will have little influence on the results, as all comparisons are relative.

Results

Variability in stomach contents

Invertebrates were numerically dominant in the stomachs of all three salmonid species (Table 1). Amphipods, primarily *Hyalella azteca*, were most abundant and found in nearly all individuals, followed by Diptera, Ephemeroptera, Mollusca, terrestrial insects, other miscellaneous aquatic invertebrate taxa, and Coleoptera. Fish remains were found in 39% of the brown trout and 22% of the rainbow trout, and deer mice were found in 7% of the brown trout sampled. Salmonid eggs were found in 1% of the brown trout and 3% of rainbow trout. No fish or fish eggs were found in mountain whitefish, and no mice were found in rainbow trout or mountain whitefish. Three percent of the brown trout, 3% of rainbow trout, and none of the mountain whitefish had empty stomachs. Brown trout and rainbow trout stomach contents were more taxonomically diverse than mountain whitefish. Based on abundance, 91% of the items found in mountain whitefish stomachs were Diptera (49%) or Amphipoda (42%). Two large brown trout (471 and 510 mm) had only fish in their stomachs. The smallest fish that had consumed a fish was a 250 mm rainbow trout (the minimum size threshold for fish used in our analysis).

Results from the MDS ordination indicated that stomach contents did not group strongly by species or year (Fig. 1), suggesting little diet specialization among species or variation in diets among years, at least in September. Note that diet specialization could not be evaluated for other months of the year, as stomach contents were only examined for fish caught in September. This pattern was also confirmed by ANOSIM. Species groups nested within years were not significantly different from each other ($R = 0.02$, $p = 0.357$; pairwise R values = 0.08–0.112, pairwise p values = 0.127–0.206), indicating that stomach contents were virtually indistinguishable between salmonids. Similarly, stomach contents were similar among years ($R = 0.02$, $p = 0.357$; Fig. 1). The greater dispersion of data (Fig. 1) for brown trout relative to the other two species is likely an artifact of the greater number of individuals evaluated (see sample sizes). SIMPER did identify some minor differences; fish and mice, for example, were more abundant in brown trout, and Diptera and Ephemeroptera were more abundant in mountain whitefish.

Diet overlap versus sample size

Schoener diet overlap values were 0.61 between brown trout and mountain whitefish, 0.78 between brown trout and rainbow trout, and 0.81 between mountain whitefish and rainbow trout, suggesting biologically significant diet overlap among all three species (Zaret and Rand 1971). Based on our random bootstrap, mean diet overlap values among all species increased with increasing sample size before reaching a plateau around $n = 10$ for all comparisons. Precision around mean overlap values was consistently less (wider 95% CI) for sample sizes less than $n = 25$ for all comparisons (Fig. 2).

Variability in stable isotopes

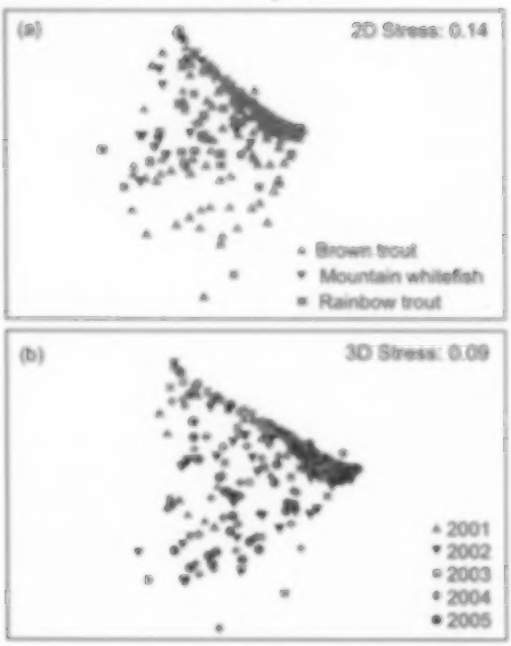
For both $\delta^{13}C$ and $\delta^{15}N$, we observed no significant variation in $\delta^{13}C$ and $\delta^{15}N$ measured from replicate muscles samples taken from an individual fish (see ±95% CI) and little

Table 1. The percent composition as occurrence (%*O*) and number of individuals (%*N*) in the stomachs of three salmonid species collected from the Green River downstream from Flaming Gorge Dam, Utah, annually in September from 2001 to 2005.

	Brown trout (n = 139)		Mountain whitefish (n = 33)		Rainbow trout (n = 111)	
Taxon	%*O*	%*N*	%*O*	%*N*	%*O*	%*N*
Ephemeroptera	41.6	6.2	60.8	7.5	50.4	8.0
Coleoptera	5.2	0.1	25.5	0.1	7.9	0.1
Diptera	79.2	10.3	100.0	49.1	96.1	20.8
Amphipoda	89.6	69.0	98.0	42.3	96.1	66.4
Mollusca	33.1	12.0	41.2	0.5	29.9	1.5
Other aquatic insects	24.7	0.7	41.2	0.5	42.5	1.0
Terrestrial insects	27.9	0.9	17.7	0.0	55.1	2.0
Fish eggs	1.4	0.1	0.0	0.0	2.7	0.1
Fish remains	39.0	0.6	9.8	0.0	22.1	0.1
Deer mice	7.1	0.1	0.0	0.0	0.0	0.0
Empty	3.2	—	0.0	—	3.1	—

Note: Data by salmonid species were pooled across years.

Fig. 1. Nonmetric multidimensional scaling ordination of diet categories among brown trout, mountain whitefish, and rainbow trout (*a*) and among 5 years of collection (*b*). Stomach contents were collected annually from the Green River downstream from Flaming Gorge Dam, Utah, in September between 2001 and 2005. Each point represents the stomach contents of an individual fish. Points close to each other represent similar stomach contents, and points more distant indicate differing contents.



variation between adipose tissue and muscle tissue. On average, the difference (absolute value) between pairwise comparisons of means among individual muscle tissue was greatest for rainbow trout (2.5‰ for $\delta^{15}N$ and 1.61‰ for $\delta^{13}C$), followed by brown trout (1.5‰ for $\delta^{15}N$ and 1.1‰ for $\delta^{13}C$) and whitefish (0.4‰ for $\delta^{15}N$ and 1.1‰ for $\delta^{13}C$). There were no significant differences among any pairwise comparison between adipose and muscle tissue for either $\delta^{13}C$ or $\delta^{15}N$ for both rainbow trout and brown trout (df = 3–7, $P > 0.05$). For mountain whitefish tissue, we observed no statistical difference in mean $\delta^{15}N$ between adipose versus muscle (df = 6, $P > 0.05$); whereas, muscle tissue $\delta^{13}C$ was ~2‰ lighter than adipose $\delta^{13}C$ on average, a difference that was statistically significant (df = 7, $P = 0.003$). However, it is important to note that the variance was extremely low among replicate samples within each category, such that very small differences could be statistically, albeit not biologically, different.

Variation among individuals and precision versus sample size

The top model for both $\delta^{15}N$ and $\delta^{13}C$ included significant effects of fish species and year and fish length for $\delta^{15}N$ (Table 2). Across all years, mean $\delta^{15}N$ was significantly greater in brown trout (17.58‰) than in rainbow trout (16.82‰), but not between brown trout and mountain whitefish (17.18‰) or between mountain whitefish and rainbow trout. $\delta^{13}C$ was significantly less in mountain whitefish (–28.89‰) than in brown trout (–26.77‰) and rainbow trout (–27.25‰), but not between brown trout and rainbow trout. The range in mean $\delta^{15}N$ among years was 1.9‰ for brown trout, 2.0‰ for mountain whitefish, and 2.6‰ for rainbow. The range in mean $\delta^{13}C$ among years was 0.7‰ for brown trout, 2.1‰ for mountain whitefish, and 1.1‰ for rainbow trout. $\delta^{15}N$ increased significantly with length for all species. Brown trout and mountain whitefish $\delta^{15}N$ increased the least, and rainbow trout $\delta^{15}N$ increased the most, with variation (increase) in length (Fig. 3), indicating that rainbow trout appeared to undergo the greatest ontogenetic change in diet even after reaching 250 mm. Mountain whitefish $\delta^{13}C$ did not vary with fish length, rainbow trout $\delta^{13}C$ became significantly more depleted with increasing length, and brown trout $\delta^{13}C$ became significantly more enriched in larger fish (Fig. 3). The overall relationships between fish length and $\delta^{15}N$ and $\delta^{13}C$ values suggest diet

Fig. 2. Mean (±95% confidence interval, CI) Schoener diet overlap values (Schoener 1970) between brown trout and mountain whitefish (●), brown trout and rainbow trout (○), and mountain whitefish and rainbow trout (▼) for different samples sizes. Diet overlap is generally considered to be biologically significant when the value exceeds the horizontal line at 0.60 (Zaret and Rand 1971). Stomach contents were collected annually from the Green River downstream from Flaming Gorge Dam, Utah, in September between 2001 and 2005. Mean and 95% CI were calculated by randomly resampling 100 times for each sample size bin.

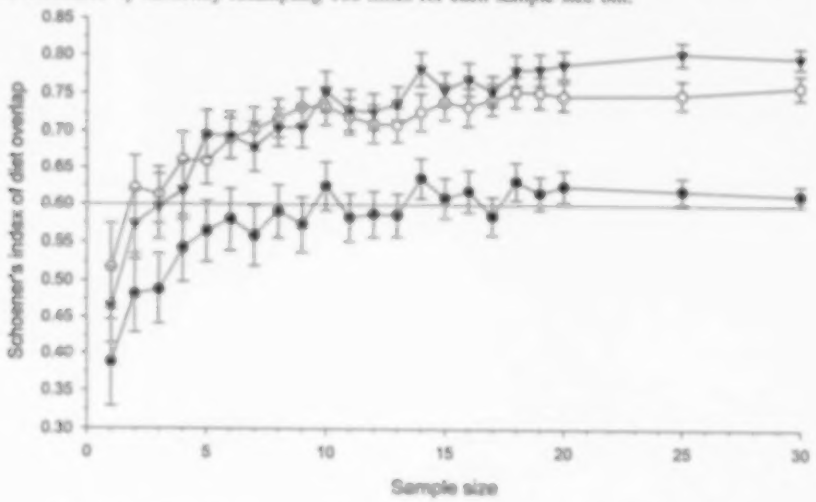


Table 2. Results for the general linear models of $\delta^{15}N$ and $\delta^{13}C$ as response variables.

Effect	Numerator df	Denominator df	*F*	*p*
$\delta^{15}N$				
Species	2	111	12.65	<0.0001
Year	4	131	39.26	<0.0001
Species × year	8	137	1.18	0.3170
Fish length	1	85.6	44.91	<0.0001
Fish length × species	2	93.1	5.56	0.0052
$\delta^{13}C$				
Species	2	89.9	14.99	<0.0001
Year	4	112.0	15.05	<0.0001
Species × year	8	131.0	1.52	0.1559
Fish length	1	72.7	0.18	0.6712
Fish length × species	2	91.1	15.30	<0.0001

Note: Fish species and year were included as categorical fixed-effects factors, and fish length was included as a centered, continuous fixed-effect factor.

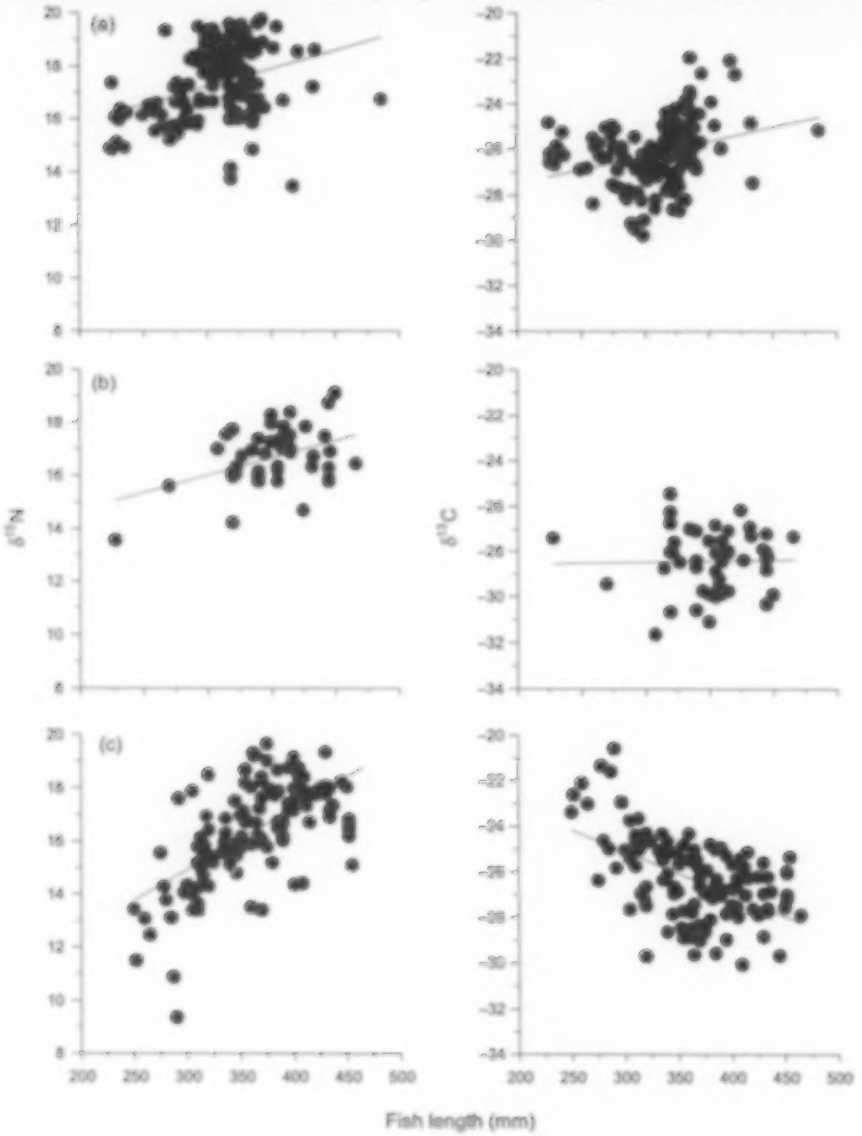
overlap was greatest between >350 mm brown and rainbow trout, and less overlap was apparent in smaller individuals.

Based on our bootstrapping simulations, the precision (95% CI) around the average $\delta^{13}C$ and $\delta^{15}N$ isotopic value increased substantially above sample sizes of $n = 10$ and reached a plateau around $n = 15$ for mountain whitefish, $n = 20$ for brown trout, and $n = 25$ for rainbow trout (Fig. 4). Precision for the average isotopic value was consistently less (wider 95% CI) for $\delta^{13}C$ as compared with $\delta^{15}N$ for all three species.

Food web and trophic overlap

Brown trout were the top predators, as indicated by their high $\delta^{15}N$ (Fig. 5). The mean $\delta^{15}N$ of mountain whitefish and rainbow trout were about 0.5–0.8‰ lighter than brown trout, respectively. The mean $\delta^{15}N$ of sculpin was 3.5‰ lighter than brown trout and 2.8‰ lighter than rainbow trout, suggesting brown trout prey heavily on sculpin and that rainbow trout also likely fed on sculpin. Mountain whitefish had similar mean $\delta^{15}N$ values as brown trout and rainbow trout, but their $\delta^{13}C$ was about 3‰ lighter, suggesting mountain whitefish rely on prey utilizing different basal resources. $\delta^{13}C$ values implied they were primarily consuming invertebrate detritivores; however, mean $\delta^{15}N$ for invertebrate detritivores was 7‰ less than mean mountain whitefish $\delta^{15}N$, indicating they were consuming and assimilating higher trophic level prey than invertebrate detritivores, such as salmonid eggs, which had mean $\delta^{15}N$ values 1.7‰ lighter than mountain whitefish (Fig. 5). Among invertebrates, predators occupied a higher trophic position than detritivores and herbivores. Mean $\delta^{13}C$ values for inver-

Fig. 3. Relationship between $\delta^{15}N$ and $\delta^{13}C$ values and fish length for (*a*) brown trout, (*b*) mountain whitefish, and (*c*) rainbow trout collected annually from the Green River downstream from Flaming Gorge Dam, Utah, in September between 2001 and 2005.



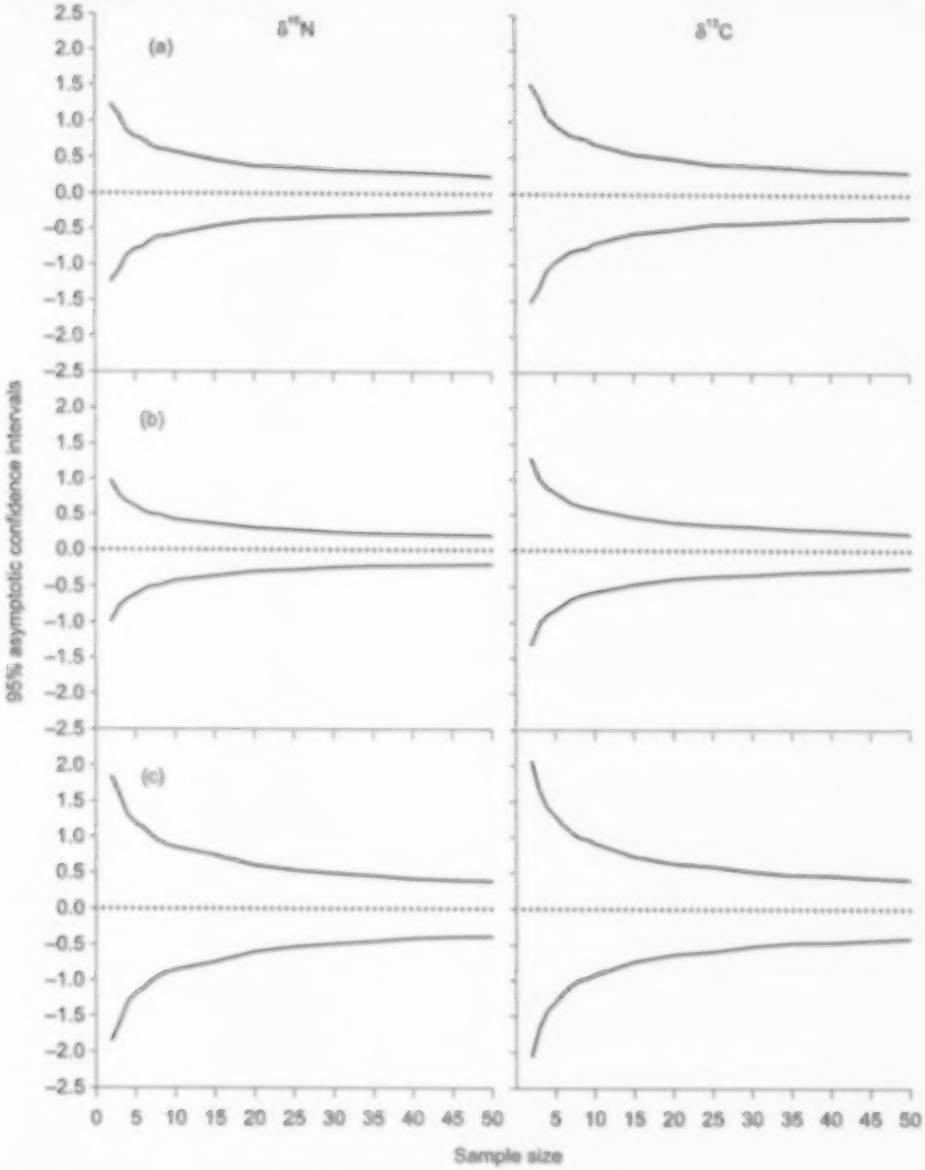
tebrate detritivores were 1.2‰ to 1.7‰ lighter than invertebrate predators and herbivores, indicating that invertebrate detritivores and herbivores were utilizing different resources.

Our analysis of the effect of sample size on trophic separation showed that major trophic level groups, i.e., predatory fish, prey fish, and invertebrates, and among salmonid species demonstrated distinct combinations of mean $\delta^{13}C$ and $\delta^{15}N$, but 95% CIs were broadly overlapping for sample sizes $n \leq 10$ (Fig. 5), suggesting little separation in dietary resources among trophic level groups when few samples were collected. Separation among trophic levels and among salmonid species increased with increasing sample size. For sample sizes $n \geq 10$, overlap in 95% CI among taxonomic or trophic groups was less evident, suggesting dietary resources became more distinct among trophic levels groups and especially between brown trout and rainbow trout when sample sizes reached $n = 25$.

Costs

Stomach content processing costs averaged \$25.49 ± \$2.91 (95% CI) and ranged from \$1.50 for an empty stomach to \$291.50 for a sample with 2330 items. The median cost was \$19.00, 36% more than the per-sample cost for determining $\delta^{15}N$ and $\delta^{13}C$ values. The average time to remove the debris and identify the organisms in a stomach sample was 1.3 ± 0.2 h (95% CI) and ranged from 0.1 to 13.5 h;

Fig. 4. The precision (95% confidence interval, CI) around mean $\delta^{15}N$ and $\delta^{13}C$ values from muscle tissue from (*a*) brown trout, (*b*) mountain whitefish, and (*c*) rainbow trout collected annually from the Green River downstream from Flaming Gorge Dam, Utah, in September between 2001 and 2005. 95% CIs were calculated using 1000 bootstraps of the data for each sample size category (increments of 1 for n = 2–10, increments of 5 for n = 10–50).



the median time was 1.0 h. The average time to enter the data into our computer program was 0.1 h per sample. Stomach sample processing costs increased sharply with the number of organisms within a stomach (Fig. 6).

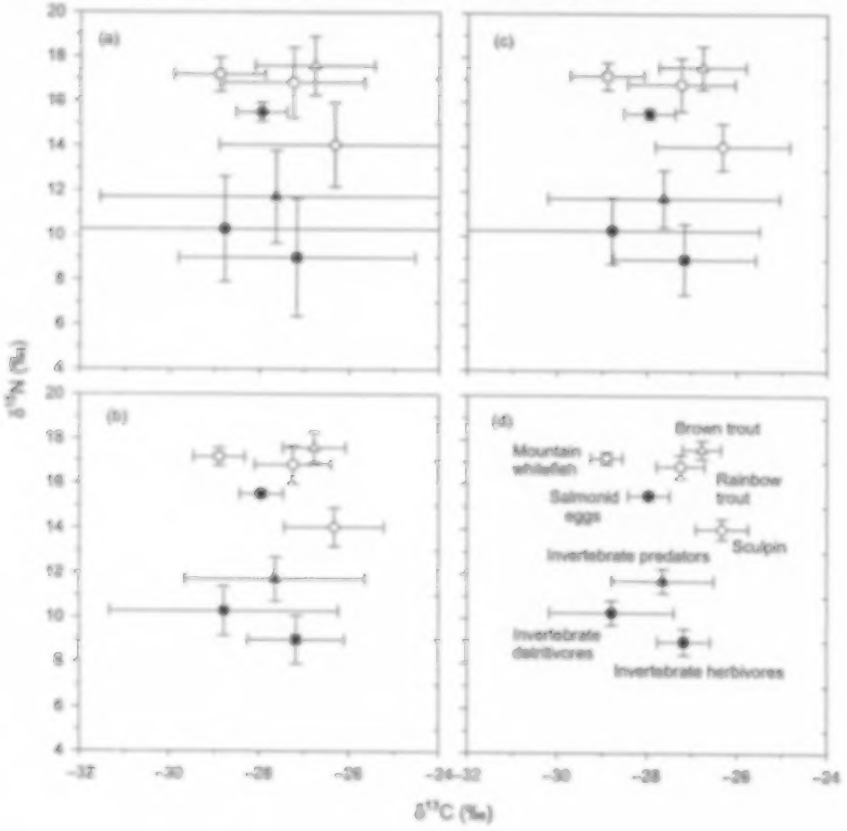
Based on our analysis of stable isotope costs and the natural variability around mean values, overall cost per analysis was minimized and precision was maximized at a sample size of n = 9 for $\delta^{15}N$ and n = 10 for $\delta^{13}C$ (Fig. 7). Precision (95% CI or standard error) increased dramatically up to a sample sizes of n = 10 (see also Fig. 4), while analysis costs increased linearly, indicating a sample size of around 10 generally provided the greatest precision for the lowest cost.

Discussion

Stomach content and stable isotope comparability

The general feeding habits of brown trout and rainbow trout were apparent using either stomach content or stable isotope data, but the overall conclusions varied when the results of each method were considered independently. For ex-

Fig. 5. Trophic structure of the Green River downstream from Flaming Gorge Dam, Utah, based on mean (±95% confidence interval, CI) $\delta^{13}C$ and $\delta^{15}N$ values derived from samples sizes of (*a*) 3, (*b*) 5, (*c*) 10, and (*d*) 25 for each trophic category. For the lower trophic levels (sculpin, salmonid eggs, invertebrate predators, detritivores, and herbivores), the mean was held constant at a sample size of 25 (as the purpose here is to show the effect of increasing precision), and the 95% CIs were calculated based on the mean standard deviation of 100 random drawn samples for each sample size. Mean and 95% CI for brown trout, rainbow trout, and whitefish are based on the bootstrap analysis. The data point symbols are the same for each plot and labeled in the $n = 25$ plot (*d*).



ample, MDS and Schoener (1970) diet overlap analyses of stomach content data both suggested there was less diet specialization and broad diet overlap among the salmonids species evaluated here and no significant differences in diet across years, at least during September. Conversely, analysis of stable isotope signatures suggested there were significant differences in species and across years, with the three species actually holding different trophic positions. These findings corroborate those of previous studies (e.g., Beaudoin et al. 1999; McIntyre et al. 2006; McHugh et al. 2008), which have generally found that among ecologically similar species, stable isotope data appears to show less dietary overlap among species than stomach content data. Clearly, when considered independently, both the implications for future study design and the ecological conclusions that one would draw from these two types of studies are complementary, yet may be substantially different.

In addition to these general differences in conclusions about feeding habitat that arise from the two different techniques, both methods provided important dietary insights that the other did not. Stomach content data provided information about trophic links that would have been undetectable if only stable isotope data had been evaluated, such as the fairly common consumption of deer mice by brown trout. Deer mice had mean $\delta^{15}N$ and $\delta^{13}C$ values of 3.69‰ and –23.96‰, respectively, which places them about four trophic levels beneath brown trout. As such, if we had not observed mice in stomachs, we would not have identified this terrestrial and aquatic food web connection. Conversely, trophic distinction, the amount of piscivory, and the relationship between increasing piscivory and fish size were much more readily observed using stable isotope data than stomach content data. $\delta^{15}N$ values for brown trout and rainbow trout were about 3‰ heavier than that observed in invertebrate-consuming sculpin and juvenile (<250 mm) trout (mean $\delta^{15}N = 14.9$, $n = 21$), suggesting these fish consume more fish than that suggested by stomach content analysis alone. The opposing direction of the relationship of fish size and $\delta^{13}C$ between brown trout and rainbow trout was intriguing and suggests that as they grow even larger than the minimum size at which piscivory begins (~250 mm), they exhibit different foraging behavior and habitat use

Fig. 6. Laboratory processing costs associated with processing fish stomach content and stable isotope samples. The cost for processing stable isotope samples was a fixed $12 per sample for sample preparation and $\delta^{13}C$ and $\delta^{15}N$ analysis costs. Costs associated with processing stomach samples varied strongly with the number of organisms present. Two out-of-range stomach sample processing costs were not shown, $231.50 and $291.50.

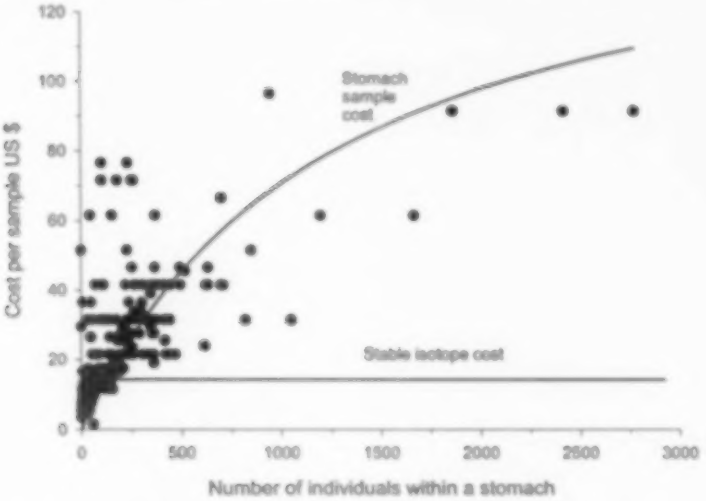
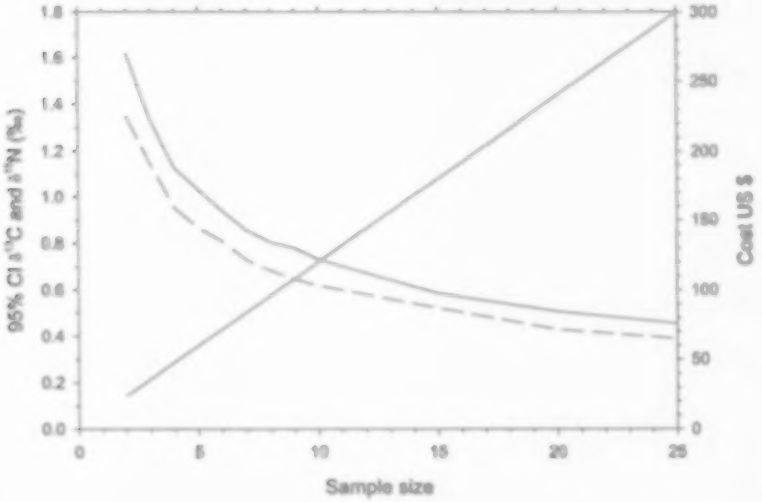


Fig. 7. The precision (95% confidence interval, CI) around pooled $\delta^{13}C$ (solid line) and $\delta^{15}N$ (dashed line) values from muscle tissue from brown trout, mountain whitefish, and rainbow trout and the costs associated with processing $\delta^{13}C$ and $\delta^{15}N$ samples (diagonal line, right y axis). Sample cost was $12 per sample for sample preparation and to obtain both $\delta^{13}C$ and $\delta^{15}N$ values.



(Newsome et al. 2009). We found these results somewhat surprising given the relative simplicity of prey resources and habitat they occupy.

Comparative results between both techniques for mountain whitefish were less consistent than for the other two salmonids species; stomach contents indicated they ate invertebrate detritivores almost exclusively, whereas stable isotope data strongly suggested they were consuming higher trophic level organisms as well. Likely possibilities for alternative diet items at higher trophic levels include salmonid eggs or small fish, yet we found no fish or salmonid eggs in their stomachs. Stenroth et al. (2006) found similar conflicting results for crayfish (Decapoda: *Pacifastacus leniusculus*); $\delta^{15}N$ and $\delta^{13}C$ values suggested they were carnivores and stomach content data suggested they were detritivores. Mountain whitefish are well known to be bottom feeders that consume aquatic invertebrates, small fish, and fish eggs (Scott and Crossman 1973). The lack of salmonid eggs in our mountain whitefish stomachs may be due to either the rapid digestion of eggs (Bailey and Houde 1989) or sample size (i.e., we evaluated too few stomachs to detect eggs), or both. This scenario provides one example of the utility of using both techniques in parallel for food web studies; future analyses can now be targeted towards determining the un-

known source of heavier $\delta^{15}N$ values in mountain whitefish diets. In sum, combining the two approaches increases the ability to interpret the overall results and led us to conclude the three salmonid species consume similar prey, but in different proportions, leading to greater niche segregation than suggested by stomach content data alone.

Variation within and among individuals and inherent limitations

We observed no significant or influential differences in isotopic composition for either $\delta^{15}N$ or $\delta^{13}C$ within individual fish or between samples collected from muscle tissue versus adipose fins. These results corroborate recent work by Sanderson et al. (2009) and Hanisch et al. (2010) and indicate it is not necessary to analyze more than one isotopic sample per individual, and that for salmonid species (and likely most fishes with an adipose fin) sampling either muscle or fins will result in the same answer. These results have obvious and encouraging implications for isotopic analyses of imperiled or protected species, as removing the muscle plugs from adult fish, or adipose fins from any age fish, is neither highly invasive nor lethal.

For studies of naturally occurring stable isotopes, variance among individuals within a population is likely the highest source of variation encountered; however, it is also a source of variation that can be controlled by adjusting study design, sample sizes, and analyses (Focken and Becker 1998; Branstrator et al. 2000). First, variation in isotopic signatures can be reduced by stratifying sampling by season or year (Vizzini and Mazzola 2003; Matthews and Mazumder 2005; this study) or habitats (Genner et al. 2003) and then by grouping age or size classes where possible post hoc (Beaudoin et al. 1999; Fry et al. 1999; this study). In contrast, although variation in stomach content samples can be minimized to some degree by sampling during times when fish are known to be feeding (e.g., crepuscular times, nonmating times), not all fish will be feeding, and many are likely to have empty stomachs or partially full stomachs (Arrington et al. 2002). The evaluation of the relative importance of individual diet items may also be skewed by both the short temporal window over which stomach content samples are generally collected and whether the sampling coincides with the occurrence of a particular prey item on the day or time the samples were collected (e.g., highly seasonal emergence event for an aquatic insect population; Ephemeroptera: Ephemeridae, or migrating terrestrial invertebrates; cicadas; Insecta: Cicadidae).

Fish collection methods may have adverse effects on stomach contents while having no effect on fish tissue used for isotope analyses. For example, gillnet-caught fish may regurgitate their stomach contents prior to collection of their stomach contents, and certain sampling techniques may be biased by collection of potentially more active, hungrier individuals (e.g., angling). It can also be difficult to identify stomach contents, especially larval or juvenile fishes and eggs, which are digested extremely rapidly (Bailey and Houde 1989; Schooley et al. 2008). Much of the evidence for piscivory in stomach content analyses is based on partially digested, unidentifiable bones that require educated guesses as to the species, size, and number of fish consumed or genetic analyses (Sheppard and Harwood 2005). The information obtained via stomach content analysis can be rewarding, but may also be difficult and time consuming to obtain.

Although generally more precise than diet data, when sample sizes are adequate, stable isotope data are also subject to their own suite of limitations and sources of variation that may be underestimated in our study. First, our study was performed in a large mountain river, typical of the Intermountain West and was characterized by a relatively simple food web (e.g., four to five trophic levels and only four species of fish). Isotopic overlap will generally increase as the number of species and trophic levels increases in more complex food webs, thus necessitating a greater sample size per species evaluated and potentially limiting our ability to infer trophic status and niche differentiation; e.g., see Szepanski et al.'s (1999) analysis of wolf diet data reanalyzed by Phillips and Gregg (2003). Although not a focus of our study, isotopic mixing models that report results in terms of the distribution of possible results can reduce the effects of these complications (Phillips and Gregg 2003). However, the typically unknown failure to include all end members, small sample sizes, and necessary assumptions regarding fractionation still limit our ability to infer trophic status and habitat use when food webs are complex and prey sources occupy numerous trophic levels. Nevertheless, additional research on species-specific isotopic turnover rates and fractionation, currently an area of considerable focus in stoichiometry and biogeochemistry, will likely improve the predictability of isotopic signature transfer between organisms.

Study design lessons and recommendations

While we provide specific recommendations on study design and sample size for ecological isotopic composition studies below, the rule for determining sample sizes should be similar to the general rule for all statistical procedures. Sample sizes must be scaled to the level of detection required to address study hypotheses; the smaller the difference that must be detected, the larger the sample size must be (Krebs 1989). These optimizations are more difficult in stomach content studies where measures of variation are difficult to acquire. Ferry and Caillet (1996) reported that none of the more than 200 dietary studies they reviewed provided any estimates of precision in describing diets. We also had difficulty in determining a useful measure of variance for our stomach content data, and in our analysis, diet overlap increased with increasing sample sizes, a trend opposite of that inferred from stable isotopes.

Our study of stomach contents and stable isotope data from the Green River is one of just a few field studies that have applied these two approaches to the same fish (Beaudoin et al. 1999; McIntyre et al. 2006; McHugh et al. 2008). Collectively, these studies have found that stable isotope data perform better at identifying trophic position and ontogenetic changes in diet, while stomach content analyses perform better at identifying specific prey species consumed. We believe the recommendations we provide below will improve future studies and increase standardization of study design with respect to sample sizes. While our analysis of 283 fish for both isotopes and diet was quite large, we note that this data was collected from a relatively simple, albeit common food web including only four to five major trophic

levels and only four species of fish; our recommendations should be taken within the context of that data set.

Based on stomach contents, even with a very large data set, we could not readily or statistically distinguish differences in stomach contents among species or years, but we did obtain a reasonable qualitative sense of which prey were being consumed by fish; this information helped elucidate some of the patterns we observed in the stable isotope analysis. Observing wide variance in diets among individuals, while not statistically pleasing, does have important implications for understanding the overall ecology of the system and population dynamics of both predators and prey. Another consideration for collecting stomach content samples will be when comparisons to historical (before-isotope) diet information are needed. By combining stable isotope and stomach content analyses, we concluded the three salmonid species consume similar prey, but in different proportions. These results support the generally held view that stable isotopes provide a time-integrated view of diets, whereas stomach contents provide a snapshot of recently digested prey and prey availability at that time. Depending on the question and the desired temporal inferences, a combination of the two approaches may be most appropriate.

Based on our bootstrapping exercise, precision in $\delta^{15}N$ or $\delta^{13}C$ increased dramatically up to a sample size of $n = 10$, with considerably less increase in precision as sample size increased further. In general, we could identify differences among all trophic levels and three ecologically similar fish species at a sample size of $n \geq 10$ and could identify significant differences at $n \geq 25$. Based on these results, stable isotope samples sizes between 10 and 25 are probably adequate for addressing most ecological questions; however, a slightly greater n may be required for addressing questions where carbon is the sole or primary focus or for species with more expansive diets, e.g., in our study, characterization of rainbow trout diets would require more samples than would mountain whitefish. Stenroth et al. (2006) recommended a sample size of 14 for detecting a 1‰ difference in isotopic values between crayfish populations. For stomach content analyses, mean diet overlap values among all three species plateaued around $n = 10$, and variation around mean values (95% CI) was consistently less (wider 95% CI) for sample sizes less than $n = 25$ for all comparisons. These results suggest that samples sizes of $n \geq 25$ are probably needed for addressing diet overlap among ecologically similar species via stomach content analysis.

In terms of cost, isotope-associated costs are known precisely prior to project implementation, whereas stomach content analysis costs vary with stomach fullness and level of taxonomic detail. In our study, isotopes averaged about one-third of the cost of stomach content analyses. The overall cost per isotope analysis was minimized, and precision was maximized, at a sample size of $n \sim 10$ for both $\delta^{15}N$ and $\delta^{13}C$. Beyond this sample size ($n \geq 10$), precision increased at a much slower rate while costs increased linearly, again indicating a sample size of about $n \geq 10$ generally provides the most precise information for the given cost. Note however, this determination will be dependent on the similarity in diet between species; for example, in our study, the ability to differentiate trophic status between brown trout and mountain whitefish would require fewer samples ($n \sim 5$) than it would to differentiate between brown trout and rainbow trout ($n \sim 25$). Lower costs associated with stable isotopes may allow for increased stratification by fish size, habitat, and season that may help explain the observed variance among individuals. Nevertheless, for stable isotopes, one also may need to collect data for prey that likely contribute to the diet, and it may be necessary to obtain these samples more than once a year, as the temporal variation in stable isotope signatures tend to increase with decreasing body size (Cabana and Rasmussen 1996). High costs associated with processing stomachs with large numbers of prey items can be reduced through subsampling, but subsampling may also reduce the ability to detect rare prey items (Vinson and Hawkins 1996). In conclusion, both approaches are complementary and appear to work best when they are combined together. Therefore, the true cost of obtaining diet information could be higher when using either alone.

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PERSPECTIVE / PERSPECTIVE

Isolation or metapopulation: whence and whither the Smith Sound cod?

George A. Rose, R. John Nelson, and Luiz G.S. Mello

Abstract: In April 1995, a spawning aggregation of "northern" Atlantic cod (*Gadus morhua*) (10 000 t) appeared in Smith Sound, Newfoundland, growing to 26 000 t by year 2000. The origin of the founder year classes (primarily the 1990 and 1992 year classes) and potential for expansion remains controversial, with genetic isolation used to justify reopenings of coastal fisheries. We investigated the origin using historical, demographic, and genetic data. History provided no evidence of large aggregations before 1995. Demographics in the early 1990s suggested few spawners in Smith Sound, but many in the adjacent Bonavista Corridor. The strong 1990 year class was not evident until 1995 and the strong 1992 year class until 1997 (both age 5 and first maturity). Genetic study of six microsatellite loci from 791 cod from overwintering aggregations in Smith Sound and offshore regions indicated little to no differentiation (F_{ST}) among southern groups (Smith Sound, Bonavista Corridor, Halibut Channel). These results provide a perspective that these groups comprise a metapopulation and that the Smith Sound aggregation arose not from self-recruitment but immigration. By 2009, the aggregation had dispersed, with large concurrent increases in the Bonavista Corridor.

Résumé : En avril 1995 est apparu dans le détroit de Smith, Terre-Neuve, un rassemblement reproducteur de morues franches (*Gadus morhua*) (10 000 t) « du nord » qui a atteint 26 000 t en l'an 2000. L'origine des classes d'âge fondatrices (principalement les classes annuelles de 1990 et 1992) et leur potentiel d'expansion sont toujours des sujets de controverse et leur isolement génétique a servi à justifier la réouverture des pêches côtières. Nous en avons recherché l'origine à l'aide de données historiques, démographiques et génétiques. Historiquement, on ne trouve aucune indication de grands rassemblements avant 1995. Les données démographiques du début des années 1990 indiquent la présence de peu de reproducteurs; mais il y avait de nombreux reproducteurs dans le corridor adjacent de Bonavista. La forte classe annuelle de 1990 n'est pas en évidence avant 1995 et la forte classe annuelle de 1992 pas avant 1997 (toutes les deux d'âge 5 et à leur première maturité). L'étude génétique de six locus microsatellites chez 791 morues provenant des rassemblements d'hiver du détroit de Smith et des régions du large montre peu ou pas de différentiation (F_{ST}) entre les groupes du sud (détroit de Smith, corridor de Bonavista, chenal Halibut). Ces résultats semblent démontrer que ces groupes forment une métapopulation et que le rassemblement du détroit de Smith provient non d'un recrutement local mais de l'immigration. En 2009, le rassemblement s'était dispersé et d'importants accroissements simultanés se sont produits dans le corridor de Bonavista.

[Traduit par la Rédaction]

Introduction

Stock structure has been a central concept of marine fish ecology and management for nearly a century (Hjort 1914; Carvalho and Hauser 1994; Smedbol and Stephenson 2001), but the factors that regulate it remain controversial. The traditional view assumed geographic stability of relatively large stock units most often based on tagging returns, with relatively "soft" but often linear boundaries among stocks (e.g., Cushing 1995). Recent studies of marine fish genetics, however, have incited a new paradigm that the scales of spatial population structure may be much finer than previously thought, with "harder" population boundaries beyond which mixing with adjacent groups was unlikely (e.g., Hauser and Carvalho 2008; Cunningham et al. 2009). This paradigm is consistent with inferences that the evolution of natal homing

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G.A. Rose[1] **and L.G.S. Mello.**[2] Centre for Fisheries Ecosystems Research, Fisheries and Marine Institute of Memorial University, St. John's, NL A1C 5R3, Canada.
R.J. Nelson. Centre for Biomedical Research, University of Victoria, Victoria, BC V8W 3N5, Canada; Institute of Ocean Sciences, Fisheries and Oceans Canada, 9860 W. Saanich Road, Sidney, BC V8L 4B2, Canada.

[1]Corresponding author (e-mail: grose@mi.mun.ca).
[2]Present address: Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada, St. John's, NL A1C 5X1, Canada.

has led to resistance to mixing, expansion, and colonization (Svedäng et al. 2007*a*). Another paradigm is based on the theory that adjacent breeding groups may comprise metapopulations within which mixing may occur either regularly or episodically and suggests that population structures are not determinants but outcomes of fish behaviour and the dynamics of the environment (Hanski and Gilpin 1997).

The geographic stability of stock structures is an assumption of most fisheries management and of increasing concern in light of the impacts of climate change on fish distributions and movement within or outside traditional stock boundaries (e.g., Nye et al. 2009; Ruppert et al. 2009). A problem in resolving such concerns is continued controversy about the enabling mechanisms involved in population structuring (Bradbury and Laurel 2007; Svedäng et al. 2007*b*; Adams et al. 2009). The controversies have typically been inspired by what may be questionable juxtapositioning rather than melding of the two broad categories of enabling mechanisms, namely, oceanographic or bathymetric containment of various life stages (Sinclair 1988; Smedbol and Stephenson 2001) and active and directed movement, largely of older fish (Harden Jones 1968; Svedäng et al. 2007*a*). As for containment and retention, at some scale it must be important to stock structure (Bradbury and Laurel 2007). For example, adults may be limited in movement by bathymetric and oceanographic (temperature or salinity) barriers (e.g., Cunningham et al. 2009), and in broadcast spawners, ocean circulation largely determines the resultant distribution of early life stages (e.g., Sinclair 1988; Nakken 2008). Nevertheless, it is difficult to conceive of how retention could act as the sole mechanism underpinning the obvious structures of many marine populations. It is likely more realistic to consider retention as setting outer boundaries within which other mechanisms, such as homing (Robichaud and Rose 2001; Knutsen et al. 2003), enable widely dispersed or mixed-stock juveniles to join reforming adult aggregations, before or at maturation, at spawning sites within the retention area (Svedäng et al. 2007*a*).

The framing of any debate about stock structure must take both temporal and geographic scales into account. At long time scales (hundreds or thousands of years), distribution patterns and stock structures are demonstrably dynamic, not static, and are determined largely by ocean climate (Briggs 1974). Retention limits can change, as can physiologically based limits to distribution, with no clearer example than the occurrence of contemporary stock structures in the North Atlantic over the same grounds covered by thick ice 10 000 years ago (Bigg et al. 2008). The genetic legacy of many species, even those having behavioural repertoires that feature strong natal homing, thus includes expansion and colonization abilities (Wood et al. 2008). For example, pink salmon (*Oncorhynchus gorbuscha*), a species known for natal homing, actively colonized much of the North American Great Lakes after a single unintended release in Lake Superior in the 1960s (Kwain and Lawrie 1981). Various introduced salmonids have colonized streams around the world, from Kenya to New Zealand, in essentially new habitats (Crawford and Muir 2008). In addition, there are numerous examples of less desirable colonizing by so-called "invasive" species (e.g., Carlton 2000). Although such examples are well known because they mostly resulted from human interventions, they demonstrate that colonizing new waters or mixing among adjacent groups is not beyond the genetic ken of many species and that any genetic propensity for phylopatry has been overcome by exceptional movements of juvenile or adult fishes. Moreover, these abilities are likely fundamental to the functioning of metapopulations (Thorrold et al. 2001), with homing and straying both enabling mechanisms of broader scale spatial population sustainability.

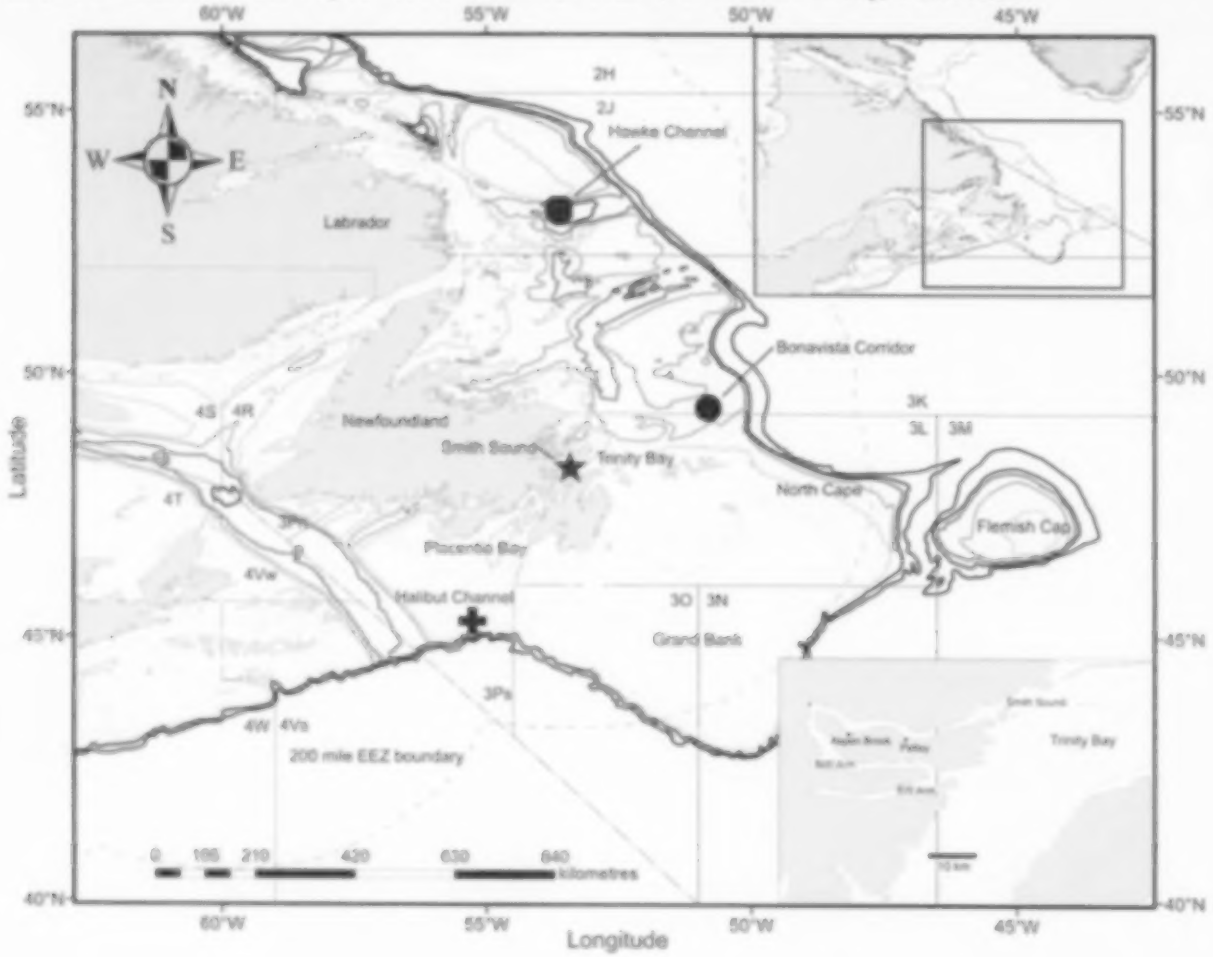
Management of fish stocks requires that stock structures and their variations with respect to ocean climate be known (Brander 2007; Allison et al. 2009) and that the factors that influence the degree of connection among spawning groups and potential for colonization of vacant or depleted grounds be better understood (Smedbol and Stephenson 2001). This is especially true for depleted stocks (McQuinn 1997; Smedbol and Wroblewski 2002; Knutsen et al. 2003), because isolated stock structures imply long-term or no rebuilding after depletion, whereas within a metapopulation, opportunistic expansion and colonization within shorter time scales is far more likely. For example, some spawning areas of Atlantic cod (*Gadus morhua*) in the Gulf of Maine remain unoccupied after many years of vacancy (Ames 1997), as have those off northern Labrador (Smedbol and Stephenson 2001), whereas other cod populations have grown substantially after being nearly extirpated (see Rose 2003 for Smith Sound cod and Mello and Rose 2009 for Bonavista Corridor cod, both Northwest Atlantic Fisheries Organization (NAFO) Divisions 2J3KL components; Gonzalez-Tronscoso and Fernandez 2009 for NAFO Division 3M cod). Other marine stocks, such as the Norwegian spring spawning herring (*Clupea harengus*), have rebuilt and recolonized old and new grounds after decades of relative stability at low stock levels (Nakken 2008). There are several hypotheses on why these differences occur. Recolonization requires a source of immigrants, which may not exist if populations were genetically differentiated or if there had been widespread stock reduction within a metapopulation. Habitat or climate deterioration can make an area unsuitable for a species, as has occurred repeatedly in the earth's history in response to changes in climate (e.g., Briggs 1974).

Largely because of these complex and often differing outcomes, the potential for expansion of extant spawning groups and recolonization of lost grounds in depleted stocks remains highly controversial, in particular for demersal species (Kura et al. 2004).

The northern cod

The "northern" cod stock complex off Newfoundland and Labrador, Canada, occupying NAFO Divisions 2J3KL (Fig. 1), was once one of the world's largest Atlantic cod stocks. This stock likely comprised over three million tonnes (Robichaud and Rose 2004) and supported a major fishery for almost 500 years. The decline of the northern cod from the 1960s to the early 1990s is well known, but details of the spatial ecology less so, despite the degree of demographic connectivity among putative groups being questioned for hundreds of years (see Rose 2007). With the advent of fisheries science (e.g., Thompson 1943), studies using tagging, morphometrics, life history comparisons, and

Fig. 1. Map showing locations of genetic samples (symbols) and places referred to in the article. Inset in upper right shows the location off the coast of eastern Canada. Lower right inset shows the Smith Sound area (the star on the main map) in more detail.



genetics have without exception described substock structure in the northern cod comprising multiple spawning components (e.g., Templeman 1974; Lear 1984; Ruzzante et al. 1999). The spawning grounds of the largest historic and latent stock components are located around the offshore banks of the Northeast Newfoundland and Labrador Shelf, with smaller components located in the coastal region (Templeman 1966). To the south, another stock complex (NAFO Subdivision 3Ps or south coast cod) migrates seasonally into the southern range of the same coastal waters occupied by the northern stock (Brattey et al. 1999; Lawson and Rose 2000) (Fig. 1). By the mid-1990s, all known stock components were at their lowest biomasses in recorded history and were put under moratoria by 1993 (Lilly 2008). The Flemish Cap cod stock, managed by NAFO, was put under moratorium in 1998.

Smedbol and Wroblewski (2002) described the substock complex of the northern cod as conforming to a metapopulation, which implies the potential for intermixing among disparate stock components. On the other hand, and in keeping with isolationist views, genetic studies indicating small genetic differences among some stock components have led to inferences that there is little potential for mixing or expansion of extant groups (most strongly stated by Beacham et al. 2002). This latter view has been influential in the northern cod being managed as multiple units, the coastal and offshore, with a fishery reopened in the coastal region but the offshore remaining under moratorium since 1992. Similar reasoning has led to the reopening of the south coast cod stock (NAFO Subdivision 3Ps) in 1997 and the Flemish Cap cod stock (3M) in 2010.

Despite these studies and management decisions, the stock structures of cod and possible demographic connections among them in Newfoundland and Labrador waters are based on a rudimentary understanding of where fish spawn, what the boundaries of retention are and how dynamic these might be, what migrations occur, and more generally, at what life stage distribution shifts or connections might occur. With the impacts of climate change perhaps already impacting cod distributions and perhaps life histories

(Rose 2005; Anisimov et al. 2007), it is imperative to better define the spatial ecology of these stocks if we are to have any hope of managing their fisheries.

Origin of the Smith Sound cod

In April of 1995, with the overall northern stock at its most depleted state, a dense aggregation of cod spanning several kilometres was observed on the bridge echosounder of the research vessel CCGS *Shamook* in Smith Sound, Trinity Bay, on the northeast coast of Newfoundland (Smedbol et al. 1998) (Fig. 2*a*). Acoustic surveys using scientific echosounders were subsequently conducted (supported by research fishing) and have been repeated in most years since this initial observation (Rose 2003). These surveys indicated that the founder year classes of the Smith Sound aggregation were spawned in 1989 and 1990 (1990 being the strongest, Fig. 3) and that these fish were reinforced by the 1992 year class, with the biomass more than doubling from about 10 000 to 25 000 t from 1995 to 2001, growing at an intrinsic rate (r) of approximately 0.2 (Rose 2003). This initial population growth rate far exceeded that of any other component of the northern cod, most of which exhibited near zero or negative growth over the same period (Lilly et al. 2004). Moreover, the high mortality evident in the offshore stock components (Lilly et al. 2006) was not evident in Smith Sound (Rose 2003). The reasons for this disparity are not known, but the Smith Sound cod quickly became the largest known overwintering and spawning aggregation within the former range of the northern cod (Fig. 2*b*).

The advent of the Smith Sound cod provided an opportunity to investigate its origins and how such a group forms. Local knowledge has been divided, with some persons believing that these fish were historically present in similar numbers, but most quite certain that they were not. The scientific data have also been equivocal.

Several previous studies have explored the relationship between cod caught in Trinity Bay and on the offshore banks using microsatellite DNA markers. Ruzzante et al. (1996) studied 334 cod collected mainly in the summers of 1992, 1993, and 1994 from Trinity Bay, including 73 fish from Smith Sound and 18 from Bonavista Bay. They found no evidence of genetic differentiation between these fish and cod caught on the North Cape of the Grand Bank from 1992 to 1994. Summer is a time of stock mixing of inshore and migrating offshore fish, hence Ruzzante et al. (1996) used assays of antifreeze protein levels in an attempt to identify fish that overwintered inshore (which were thought to have higher levels). The subsample of cod with elevated antifreeze showed very small genetic differences in microsatellite loci from the North Cape fish in some but not all tests for population structure, making the suggestion of population differentiation equivocal. Moreover, more recent studies have indicated that the use of an arbitrary antifreeze level as indication of inshore residency is questionable. In the spring of 2003, fish that had very likely overwintered inshore, based on acoustic monitoring throughout the winter and spring, showed no elevated levels of antifreeze in their blood (Lilly et al. 2004). This result casts doubt on the method used by Ruzzante et al. (1996) to distinguish among putative geographically distinct groups. Moreover, Beacham et al. (2002) detected no difference in microsatellite DNA loci between fish from Trinity Bay (collected in spring and summer 1997, 1999) and the northern region of the Grand Bank (collected summer and fall 1998, 1999). In a following study, Ruzzante et al. (1997) analyzed cod samples collected in Smith Sound in winter (50 fish) and spring (100 fish), 1995. By pooling these samples, a difference between them and the earlier North Cape sample was reported based on the genetic estimator, F_{ST}, being of marginal statistical significance (0.0014; $P = 0.049$), while another indicator, R_{ST}, was not statistically significant.

In terms of the origin of the Smith Sound cod, all three of the aforementioned studies (Ruzzante et al. 1996, 1997; Beacham et al. 2002) provide useful but problematic information. Although the location of the inshore samples is fairly well established with respect to the Smith Sound fish (especially those of Ruzzante et al. 1997), there is no such certainty about the relationship of the offshore samples taken from the northern Grand Bank to the known aggregations of spawning fish documented in 1990 and 1992 in the Bonavista Corridor (e.g., Rose 1993), a putative distant source of recruits. From first principles, oceanographic and early life history drift patterns (e.g., Pepin and Helbig 1997) are inconsistent with a northern Grand Bank source of juveniles in Trinity Bay. Also, the coastal cod used by Ruzzante et al. (1996) were collected before the Smith Sound aggregation was formed and cannot be assumed to represent the fish in question. None of these studies included fish from further south.

Of importance to management has been the potential of these fish to act as a source for early life stage expansion or juvenile–adult colonization of adjacent but depleted ranges. Again, both traditional and scientific knowledge has been divided on this issue. Beacham et al. (2002) presented the strongest view that these fish were a genetically isolated unit and that expansion was unlikely, but the opposing view that the northern cod form a metapopulation implies that expansion and repopulation of other grounds is possible at time scales of generations, despite small genetic differences (e.g., Smedbol and Wroblewski 2002). This controversy is entangled in current management, in which the formerly unified stock is now assessed and managed as two units: the inshore (where fisheries have reopened) and the offshore (which remains under moratorium) (Brattey et al. 2009). There is concern that such management may impede stock rebuilding (Shelton et al. 2006), a factor now more important because the Bonavista Corridor fish are migrating shoreward in summer as they did historically (Brattey et al. 2009). There is also a concern with fisheries open since 1997 in the adjacent south coast stock (NAFO Subdivision 3Ps) and now in NAFO Division 3M (NAFO 2009).

In this paper, we investigate how a supposedly "new" group of spawning fish, here the Smith Sound cod, might form. The question is whence, or "from what place or origin or source, or wherefrom" (http://dictionary.canadaspace.com), did they arrive in Smith Sound. We also investigate the likelihood that this group has potential to expand its range or colonize new or depleted grounds (whither or "to what place or whereto"). In essence, is the Smith Sound cod a reproductively isolated and self-recruiting population or part of a metapopulation? The most parsimonious explan-

Fig. 2. Echograms showing cod in springtime during (*a*) first survey of Smith Sound in 1995, (*b*) in 2000 at the height of its abundance, and (*c*) in 2009 with few relatively few cod remaining. All echograms are over the same grounds with total depth of about 210–230 m and spanning about 1000 m. 1995 and 2009 data were collected with a BioSonics DT system at 120 kHz; in 2000 a Simrad EK500 at 38 kHz was used.

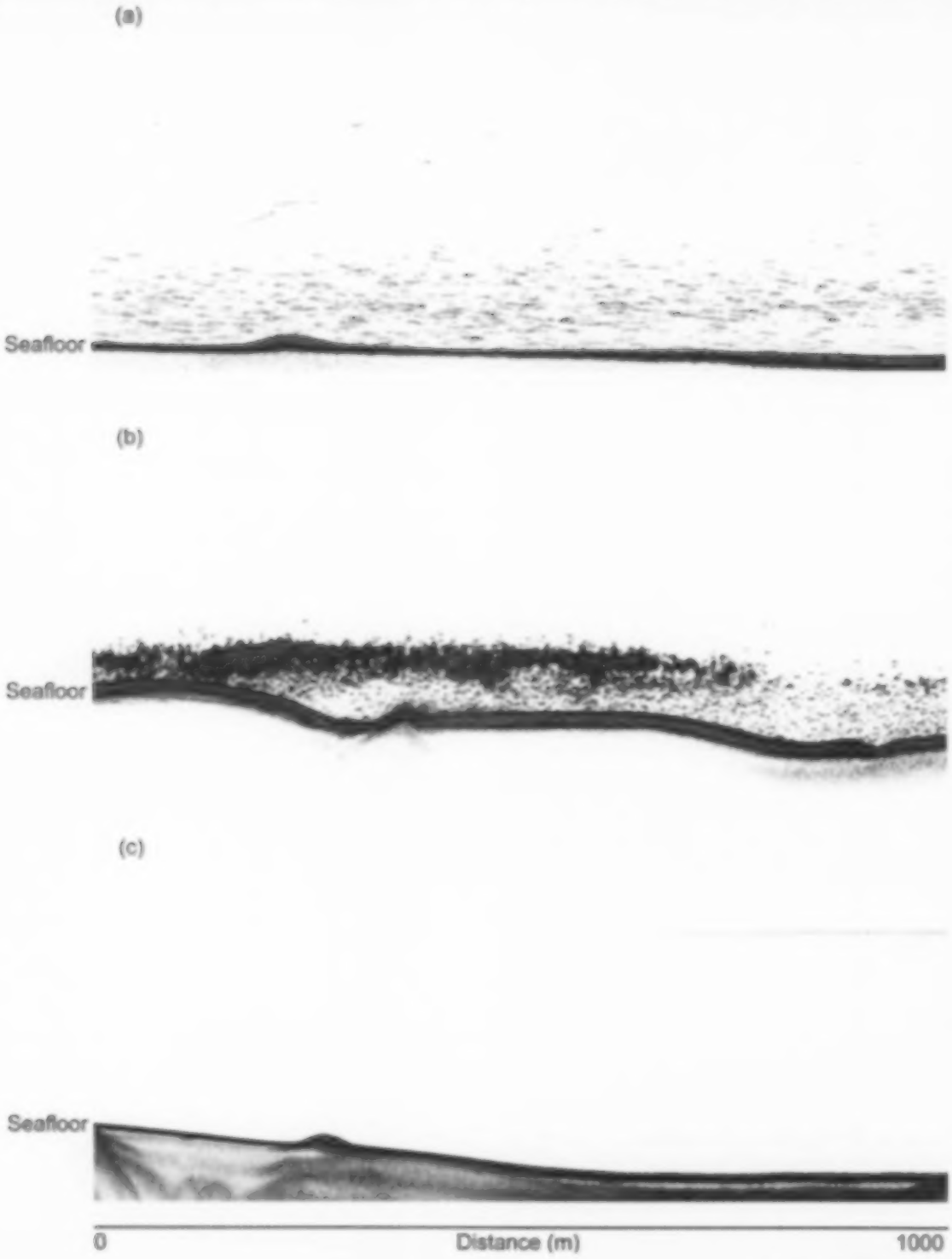
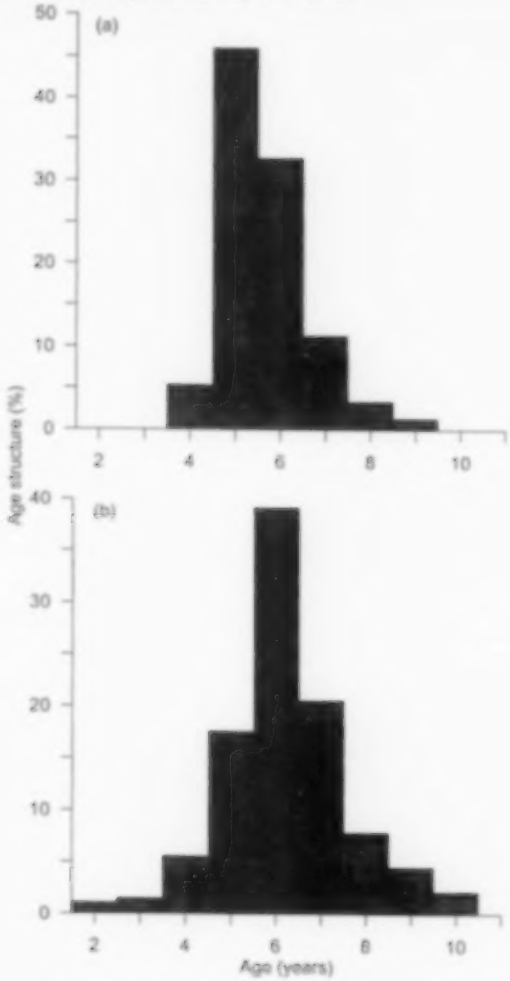


Fig. 3. Age structures from Smith Sound: (*a*) from 150 otolith readings of fish sampled during surveys in 1995; (*b*) from 741 tagged cod (age keyed from length) in 1996.



ation of the origin of the founder year classes would be a local spawning stock and is the point of departure for this study. We attempt to answer a series of relevant questions, considering historical, ecological, and genetic perspectives. We aim to use this case study to shed light on the particular situation of the Smith Sound and northern cod but also on other overexploited fish stocks that have potential to rebuild.

Data

Historical descriptions of Smith Sound and its fishery were taken from the literature and interviews with knowledgeable local persons and long-time residents. Unpublished trip reports from all cod research cruises undertaken in Trinity Bay by the Department of Fisheries and Oceans (DFO; now Fisheries and Oceans Canada) and the Fisheries Oceanography group at Memorial University from the early 1990s and up to 1995 were used to document the scientific perspective on cod in Smith Sound at that time.

Acoustic surveys and supporting research using calibrated scientific echosounders and biological sampling of cod using research trawls and handlines have been conducted in Smith Sound in winter and spring most years since 1995 using several vessels (Rose 2003, 2007). Following earlier research and acoustic surveys in the Bonavista Corridor and Hawke Channel in the early 1990s (e.g., Rose 1993; Rose et al. 2000; Anderson and Rose 2001), similar work was conducted from January to March 1998 to 2003 along the shelf edge where cod are known to overwinter in these regions and further south in NAFO Division 3Ps, both inshore (Placentia Bay) and offshore (Halibut Channel – St. Pierre Bank). More complete descriptions of the most recent offshore work, as with the earlier work conducted from the CCGS *Teleost* using calibrated 38 kHz echosounders and the Campelen 1600 research trawl, is in Mello and Rose (2008, 2009). From 1998 to 2003, a total of 16 693 cod from these regions were sampled for maturity state (Morrison 1990) and aged using otolith techniques by Norman Batten, St. John's, Newfoundland, including 4600 from Smith Sound.

In January of 2001 and 2002, fin clips were taken for genetic analysis from 791 cod from overwintering aggregations in the heart of the Bonavista Corridor and Smith Sound, and in the Hawke and Halibut channels (Fig. 1; Table 1). Approximately equal numbers of randomly selected fish from the catches were sampled from 10 cm length groups (e.g., 21–30 cm, 31–40 cm) spanning the length range of fish caught in each region, with fin clips immersed in 95% ethanol for storage. At University of Victoria laboratories (Victoria, British Columbia), six nuclear microsatellite loci (*Gmo1*, *Gmo8*, *Gmo19*, *Gmo34*, *Gmo35*, and *Gmo36*) were assayed following Beacham et al. (2002). Initial exact tests on resultant data to assess Hardy–Weinberg expectations (HWE) were performed using GENEPOP, adjusting the α value for rejection of the null hypothesis (H_0 = Hardy–Weinberg proportions) to $0.05 \times 6^{-1} = 0.0083$, using GENEPOP v. 3.1 (Raymond and Rousset 1997). Two deviations from HWE were noted within the Hawke Channel and Smith Sound samples; one was noted in the Bonavista Corridor but none from the Halibut Channel samples. Locus by locus tests of genetic homogeneity between the 2001 and 2002 samples from Smith Sound and Bonavista Corridor were carried out to determine if pooling was justified to increase the power of the analysis, with 10 000 steps in the Markov chain, 1000 "dememorization" steps, and the adjusted α value of 0.0083 (Rice 1989). No single locus test suggested rejection of the null hypothesis of genetic homogeneity, and hence the 2001 and 2002 samples were pooled by site for further analyses. The same procedure was used to test for genetic homogeneity between all samples. Pairwise F_{ST} values were calculated using GENETIX v. 4.05.2 (Belkhir et al. 2004). Potential groupings were also tested for without presupposing an origin using STRUCTURE (Pritchard et al. 2000).

Historical context

Settlement in Newfoundland from the 17th century onward occurred almost exclusively along the coast to access cod fishing grounds (see Rose 2007). Of particular note,

Table 1. Location and date of Atlantic cod sampled for genetic analyses.

Locale	Date	*n*
Bonavista Corridor	Jan. 2001	93
Hawke Channel	Jan. 2001	77
Smith Sound	Jan. 2001	123
Bonavista Corridor	Jan. 2002	200
Smith Sound	Jan. 2002	198
Halibut Channel	Jan. 2002	100

there were no fishing outports in Smith Sound as late as the mid-19th Century despite settlement being well established in this region by this time (Martin 1990). As European settlement took place in the late 19th century, the primary activities were timber cutting and agriculture and remain so today. Until the mid-1980s, the few fishermen who settled in Smith Sound were of two types: those who voyaged to Labrador each year to fish cod and those who stayed home to harvest Atlantic herring (*C. harengus*), lobster (*Homarus americanus*), and snow crab (*Chionoecetes opilio*), along with some cod (Tom Clenche, mayor of Petley, Smith Sound, Newfoundland, A0C 1P0, personal communication, 2009). While the Labrador fishermen generally prospered, those that remained in Smith Sound were generally poor (Tom Clenche, personal communication, 2009). With the arrival of gill nets in the early 1960s, many small coastal groups of fish were fished to commercial extinction, including the cod in Smith Sound (Les Dean, former Deputy Minister, Department of Fisheries and Aquaculture, Newfoundland and Labrador, with family heritage in this region, personal communication, 2009). The major fishing of Smith Sound apparently took relatively few fishermen only two or three seasons, suggesting that the local cod group likely comprised only a few thousand tonnes. From the mid-1960s until the mid-1980s, local fishermen returned to fishing herring, lobster, and snow crab, supplemented with migrating cod from the offshore banks.

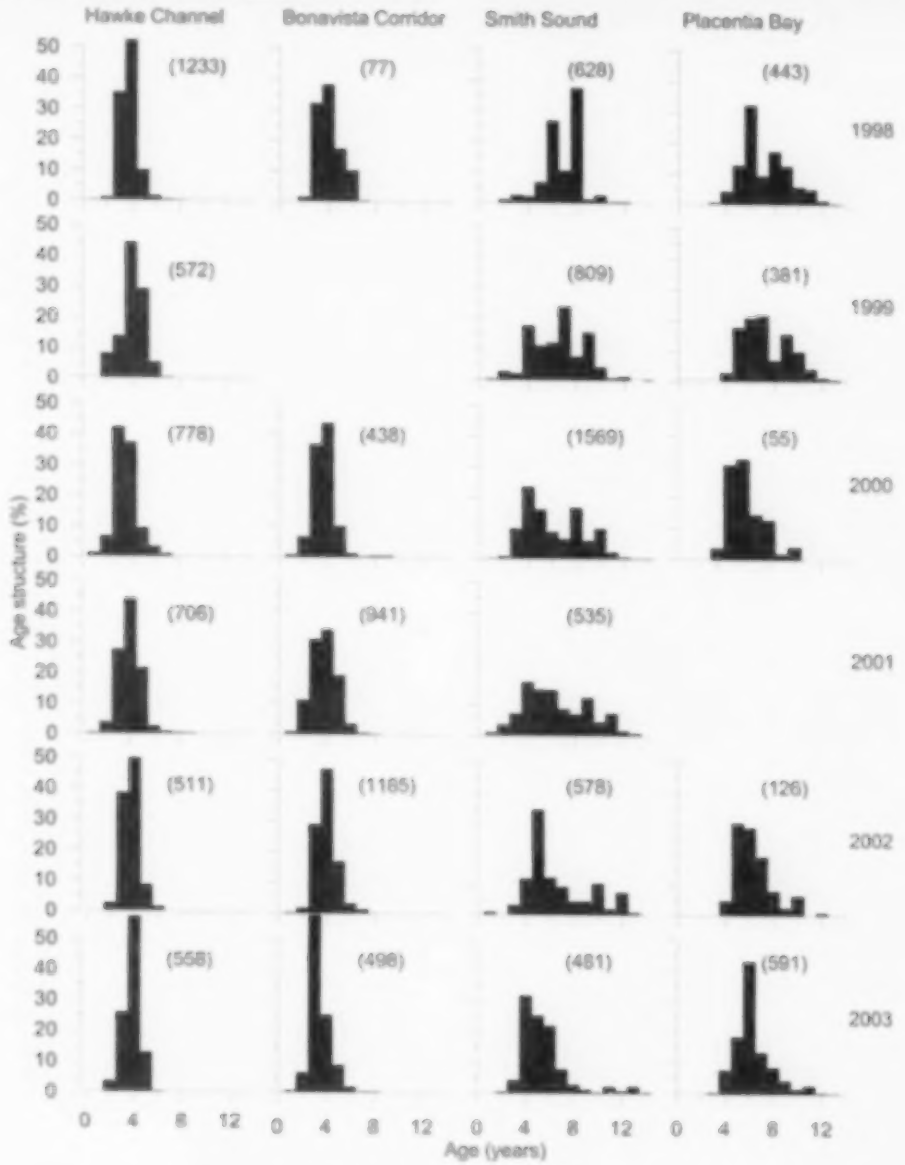
Considering inner Trinity Bay, including Smith Sound and adjacent areas close to Smith Sound, there is little evidence of spawning fish during this era. Tagging studies in June of 1981 and 1982 conducted by DFO released 3992 cod, not one of which was >45 cm in length, nor likely mature (data in Taggart et al. 1995). Although the bulk of recaptures of those fish occurred within Trinity Bay, 20%–30% of these fish exhibited what the authors called "directional travel", with a portion of those caught offshore, and particularly in the Bonavista Corridor, in subsequent spawning seasons. As these tagging studies were conducted in June, it is possible that Bonavista Corridor onshore migrants were among those tagged, but it is more likely given their size that they were juveniles yet to conduct their first spawning migration. This in turn suggests that juveniles that would eventually spawn both inshore and offshore were mixed within Trinity Bay, at least up until age 4. In those same years of 1981 and 1982, 5643 cod were tagged in the Bonavista Corridor region, of which >90% were >50 cm in length, with fish >75 cm common (this does not represent overall age structure, as smaller fish would neither have been caught nor tagged in equal proportion to larger fish, but it does indicate the presence of large numbers of spawning fish; data from Taggart et al. 1995). Beginning in the late 1980s, in keeping with a distribution shift in the dominant bank groups of adult northern cod from north to south (deYoung and Rose 1993; Rose et al. 2000), the situation of the Smith Sound fishermen was reversed, with the Labrador fishery failing but the southern fisheries (including Trinity Bay) assuming a new if short-lived prosperity right up until the final decline of the stock (Denis Ivany, commercial fisherman, Petley, Newfoundland, A0C 1P0, personal communication, 2009). It is unlikely that the source of this fleeting abundance of fish was local, as the fishery was seasonal from spring to fall in keeping with known migrations from the Bonavista Corridor in those years (Rose 1993), whereas the migrations of the Smith Sound cod are quite different, taking them out of the Sound and frequently out of Trinity Bay from summer to fall (Brattey and Healey 2005).

Was the cod aggregation present prior to 1995?

Trip reports from DFO research cruises, whose objectives were to locate cod for tagging and other research, suggested that few cod were present in Smith Sound in the early 1990s other than during the offshore migration. On 8 June 1993, at a time when some overwintering cod would be expected to remain aggregated in Smith Sound (Rose 2003; Brattey et al. 2008*a*), the trip report states that only two cod were caught and that snow crab pots were ubiquitous. The presence of snow crab may have hindered cod fishing but is a further indication of few cod, as snow crab is a frequent diet item of larger cod in this region (Sherwood et al. 2007). After 1995, with the increase in cod, the snow crab fishery in Smith Sound ended abruptly for lack of catch (Denis Ivany, commercial fisherman, Petley, Newfoundland A0C 1P0, personal communication, 2009), despite rapid increases in crab numbers during these years over most of the former range of the northern cod (Dawe et al. 1997). In keeping with this local knowledge, remotely operated vehicle observations of former crab grounds within the Sound in the early 2000s failed to find even a single crab (G. Rose, unpublished data). In June of 1994, just 1 year prior to the presence of the large and conspicuous aggregation, some cod were present and caught by DFO researchers, but a tow made off Petley (a hotspot for cod after 1995) again caught only two fish. Echosounder searches for cod during these trips located very few or no fish, which contrasts with the much evident "marks" on any type of sounder after 1995 (Fig. 2; Denis Ivany and Lloyd Phillips, commercial fishermen, Petley and Aspen Brook, Newfoundland, A0C 1P0, personal communications, 2009).

There is also evidence from cod egg surveys conducted in June and July in the early 1990s. Smedbol et al. 1998 reported that egg densities increased by more than an order of magnitude in 1995 as compared with 1991 and 1993. Although there was no sampling in Smith Sound until after the aggregation was surveyed in 1995, egg densities at three stations in June ranged from 0.39 to 1.45 eggs·m^{-3}, the highest recorded in that or any other year of their data; similar results were found after more extensive sampling conducted throughout June and July 2006 and 2007 across 10 stations in Smith Sound (2006: mean = 0.47, standard deviation

Fig. 4. Age proportions of cod caught during acoustic surveys and research in the Hawke Channel, Bonavista Corridor, Smith Sound, and Placentia Bay from 1988 to 2003. *n* is in parentheses.



(SD) = 0.61, *n* = 70; 2007; mean = 0.74, SD = 0.63, *n* = 90; data from Knickle and Rose 2010).

An analysis of the tagging data from the early 1990s in Trinity Bay concluded that fishing mortality (*F*) was in the range of 1–1.5 in the early 1990s and that the local cod had been reduced to a remnant state earlier than in the adjacent offshore area (Myers et al. 1997), consistent with the local knowledge reported earlier. In contrast with the relatively few mature fish found and tagged in all of Trinity Bay in the spring and fall of the early 1990s (the length at 50% maturity was about 55 cm in 1990, from Olsen et al. 2004), over 14 000 mostly adult cod were tagged in those same years in a few days in the Bonavista Corridor with similar effort (Fig. 4). Moreover, tagging experiments in Smith Sound conducted in the late 1990s, conducted using the same vessel and gear as in the early 1990s, primarily during the spring before any onshore migration would have occurred, caught and released many thousands of adult-sized fish with similar effort as expended during the earlier era (e.g., Brattey and Healey 2005).

Overall, the weight of the historical evidence suggests that while cod were present in Smith Sound prior to 1995, as they were in most all of the bays, fjords, and inlets of the northeast coast of Newfoundland, there was no excep-

tional overwintering or spawning concentration there, especially not in the early 1990s, as was present from 1995 to the early 2000s (*e.g.*, Fig. 2*b*). Hence, any support for the hypothesis that the founder year classes of the 1995 Smith Sound aggregation were locally produced, even within Trinity Bay, must assume that a very small spawning biomass resulted in relatively strong recruitment in several years, which is atypical for northern cod (Cushing 1995). The alternative is a spawning source outside Trinity Bay.

When did the fish arrive?

In the spring of 1995, about 10 000 t of cod were surveyed within Smith Sound in a large and semipelagic distribution (Fig. 2*a*). Exactly when these fish entered the Sound is not known, but on 19 October 1994, there were few fish in what would become the centre of cod distribution near Petley, as researchers on the CCGS *Shamook* "made a tow off Petley in the afternoon in about 180 m and caught a quantity of flounder but only 5 small cod in the 20–29 cm range. Two more tows were made in about 80–115 m and 19 cod were obtained for sampling" (George and Bailey 1994). The next day, about 50 cod and "lots of flounder" were caught in two tows made several kilometres further in the Sound (near Aspen Brook) and about 70 cod were caught on handlines. These meager catches suggest that the aggregation had not arrived by late October 1994.

On 24 April 1995, it was reported from the CCGS *Shamook* on a steam through Smith Sound that "All the way in we were seeing spots on the sounder which appeared to be herring ... at 1700 [h] we made a 20 min. tow in Aspey [sic] Brook area in about 85 fath [153 m]; there were marks on the sounder which we took to be herring because of the density. When the net was retrieved, we had about 5000 lb [2200 kg] of cod [this would have been at least 2000 fish] and about 100 lb [40 kg] of [American] plaice [*Hippoglossoides platessoides*]" (George et al. 1995). Based on these reports, it appears that a large group of cod entered Smith Sound sometime between the end of October 1994 and April 1995 (see Fig. 2 from 4 May 1995).

The aggregation of cod surveyed in Smith Sound in the spring of 1995 was dominated by 5- and 6-year-old fish (1989 and 1990 year classes) (Fig. 3). These year classes, particularly 1990, remained strongly represented throughout the 1990s and early 2000s (Fig. 4). The 1992 year class was also relatively strong, but subsequent year classes up to 1996 have been weaker. The 1989, 1990, and 1992 year classes were also relatively strong further south (e.g., NAFO Division 3Ps, including Placentia Bay and Halibut Channel; Fig. 4) and on the Flemish Cap (Gonzalez-Troncoso and Fernandez 2009), but nonexistent in the Bonavista Corridor and further north in the Hawke Channel (Fig. 4).

Whence the fish?

A fundamental question is where did the founder year classes of cod come from prior to entering Smith Sound in the spring of 1995 at ages 6 and 5? There are two parts to an answer, working backwards: (*i*) Where did they spend their juvenile life? (*ii*) Where were they spawned? The most parsimonious explanation to the first query is that as

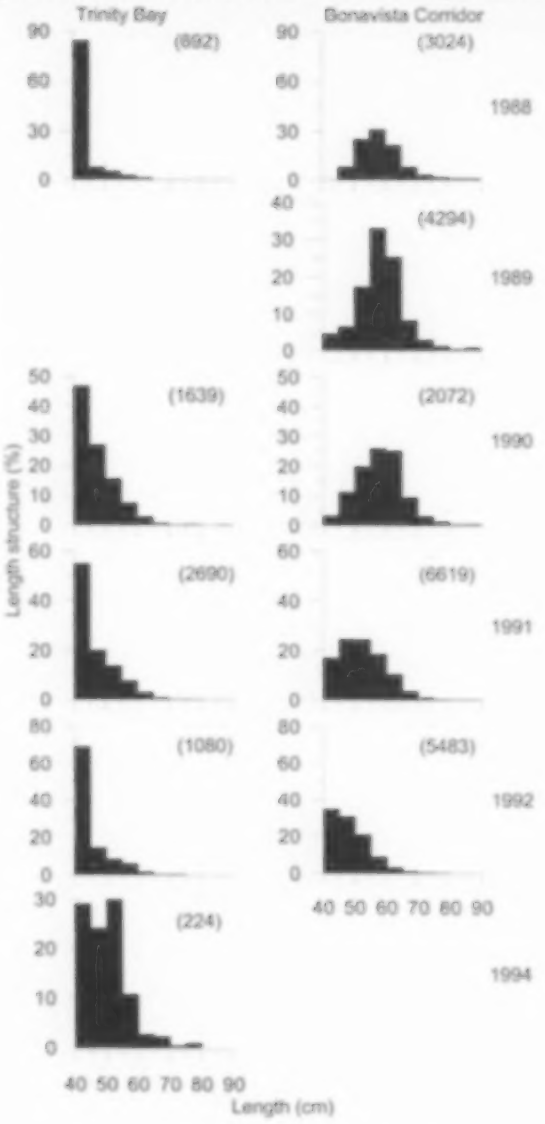
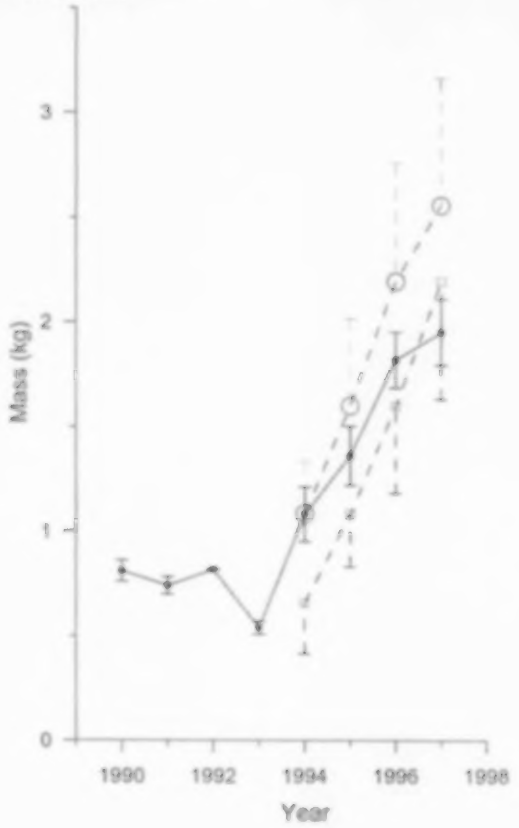


Fig. 5. Length proportions of cod caught during tagging in Trinity Bay and the Bonavista Corridor 1998 to 2004. *n* is in parentheses.

juveniles they resided in Smith Sound or the adjacent waters of Trinity Bay. Evidence from tagging research in the early 1990s suggests that there were substantial numbers of juvenile fish (lengths < 50 cm) in western Trinity Bay in the early 1990s (Fig. 5). For years when the founder year classes should have been present and for which data exist, specifically 1992 and 1994, there is evidence of numbers of 1989 year class cod that were age 3 in 1992 and age 5 in 1994. There is much less evidence of 1990 year class juveniles, which would not have been caught in 1992 but would have been in 1994 at age 4. Their low proportion in the tagged fish in 1994 but dominance in later surveys suggests they may not have been abundant in Trinity Bay until 1995. It is noteworthy that although both the 1989 and 1990 year

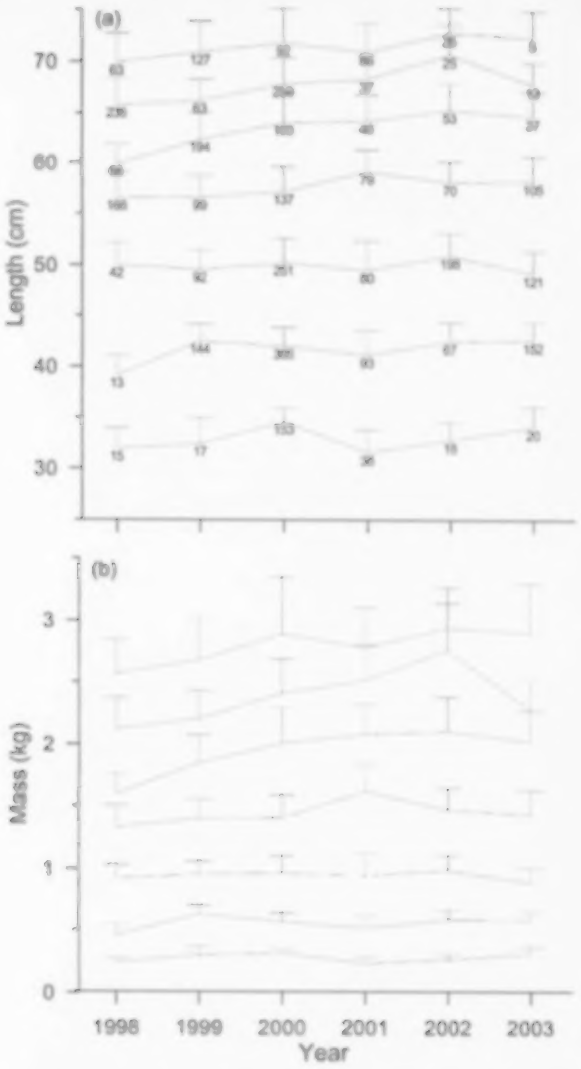
Fig. 6. Mean masses (with standard error, SE) of cod caught during tagging in western Trinity Bay from 1990 to 1997 (solid squares), and mean masses of 1989 (open circles) and 1990 (open squares) year classes based on mass at age data from cod in Smith Sound from 1998 to 2003.



classes are considered to be founders of the Smith Sound aggregation, the 1989 year class receded in importance relative to the 1990 year class as the years progressed (Fig. 4).

A comparison of the mean masses of fish tagged in the 1990s in Trinity Bay is also informative (Fig. 6). After several years of little change, the mean masses increased steadily beginning in 1994, consistent with tracking one or two year classes. In 1994, however, the mean mass of fish tagged was greater than the mean mass of 4-year-old fish (1990 year class) based on measures in Smith Sound from 1998 to 2003, but nearly identical to that of 5-year-olds (1989 year class) (Fig. 6). From 1995 to 1997, however, the mean mass of tagged fish declined relative to the estimated mass of a 1989 year class fish, approaching that of the 1990 year class. These data suggest that in 1994, 1989 year class fish were common in Trinity Bay, but beginning in 1995 the 1990 year class became dominant, which is consistent with the survey data from 1995 onwards. An argument might be made that the under-representation of the 1990 year class in 1994 was a result of lower availability to the capture method or to tagging, but this is not supported by the tagging data in other years, in which cod <45 cm in

Fig. 7. Length (*a*) and mass (*b*) of 3- to 9-year-old cod in Smith Sound from 1998 to 2003. Numbers (*n*) are shown in panel (*a*).



length (and most likely age 4 (see Fig. 7), as cod <40 cm would not have been tagged to any extent) were in some years the dominant catch and tagged size (Fig. 4). In addition, there may have been some decline in growth in length from the early to late 1990s, which would affect these interpretations, but any influence was likely to be small in 3L (Olsen et al. 2004). Overall, there appears to be reasonable evidence that of the founder year classes, the one for 1989 was well represented as juveniles within Trinity Bay, but not so the year class for 1990, which may have arrived in 1995.

There is also evidence that the 1992 year class, which bolstered the Smith Sound cod for over a decade, was not present until at least age 5. There is no evidence of the 1992 year class in the surveys of 1995, but these fish would

only have been age 3 at that time and may not have been with the main aggregation that was sampled (Fig. 3*a*). It is more difficult to explain the April 1996 tagging results from Smith Sound, which also show very few 1992 year class fish (Fig. 3*b*). At age 4, a dominant year class, as the one for 1992 turned out to be in Smith Sound, should have been evident in the tagging data if it was present. Tagging results from other areas of western Trinity Bay were similar (not shown). By the winter of 1998, however, the 1992 year class (at age 6) was prominent, as it has been since then (Fig. 4). These results raise the possibility that like the 1990 year class, the one for 1992 may have immigrated to Smith Sound sometime in 1997 at age 5. An obvious question then becomes why would they have migrated, presumably north, in those years? These year classes were relatively strong to the south (Fig. 4) prior to the reopening of the NAFO Subdivision 3Ps fishery in 1997, and that could have played a role, but it is also possible that the warming ocean conditions from 1995 to 1997 (see DFO 2009) influenced northward movement. It should be borne in mind that although precise biomass estimates for the southern area are not available as a consequence of highly variable surveys, it is likely that the numbers of fish that would have migrated north would have been a modest percentage of the total stock (perhaps 10%–15%, based on an immigration of about 6000 t from a stock that was likely of the order of 100 000 t).

The second part of the question is where the founder year classes were spawned. There were no surveys of cod in Trinity Bay in any of the years that the founder year classes were produced, so there is less evidence to summarize. Nevertheless, tagging studies indicate there were relatively few large fish in Trinity Bay compared with adjacent waters (Figs. 5, 6). According to data in Olsen et al. (2004), cod in this region would not have matured in 1989 and 1990 until >50 cm in length (50% maturing by 55 cm and >1.4 kg). Based on this maturity schedule and the tagging data, very few mature fish were present in Trinity Bay in the years in which the founder year classes were produced.

If the Smith Sound cod did not self-recruit, but form part of a metapopulation, then the founder year classes could have been spawned elsewhere, most likely in an adjacent group. During the years the founder year classes were produced, the last large spawning aggregations of northern cod (~450 000 t in 1990 and 150 000 t in 1992) were located within the Bonavista Corridor, a constricted basin that extends over 150 km from the shelf break to the coast and directly into Trinity Bay (Rose et al. 2000). These fish migrated shoreward while spawning en route in both these years (Rose 1993). The major juvenile grounds for these northern cod are thought to be coastal waters that include Trinity Bay (Dalley and Anderson 1997); hence, it is not unreasonable that recruits from Bonavista Corridor spawning could have contributed to the Smith Sound aggregation as they matured in the mid-1990s (e.g., Taggart 1997). No assumption of an atypical egg or larval drift pattern is necessary under this hypothesis, although an assumption must be invoked that the ontogenetic migration of prespawning 3- to 4-year-olds back to their natal area did not occur (see Dalley and Anderson 1997). This is not an unreasonable assumption, given that the Bonavista Corridor region was near de-

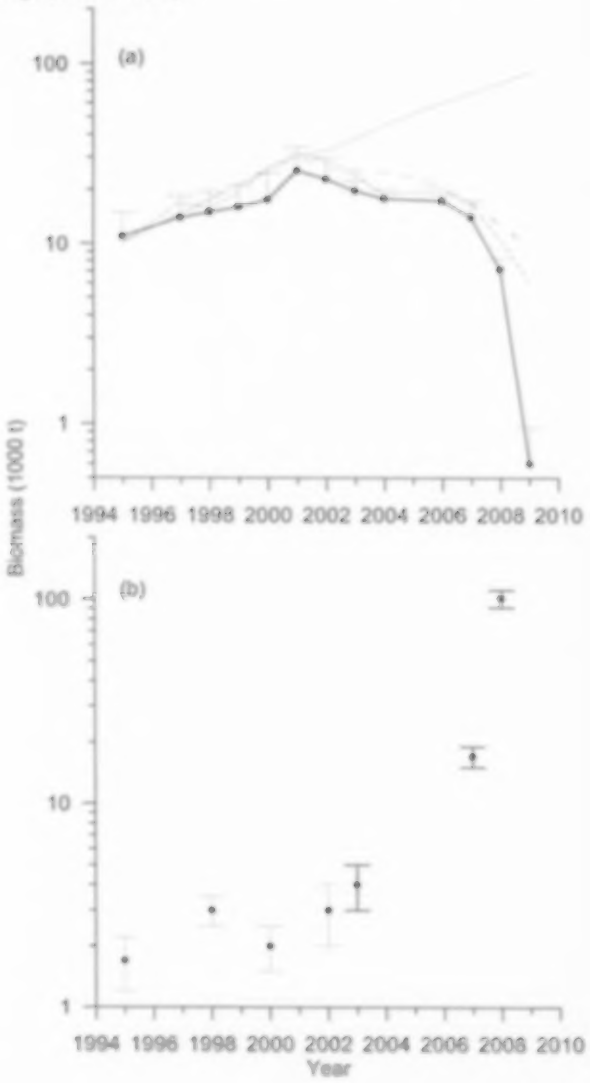


Fig. 8. (*a*) Cod biomass during the overwintering-spawning period measured by acoustic surveys in Smith Sound from 1995 to 2000 (closed circles showing 95% confidence intervals, CIs), biomass plus catch (lower solid line), estimate of abundance assuming zero growth and fishing (broken line), and growth at the rate estimated from 1995 to 2001 (18% per year) with fishing (upper solid line). (*b*) Cod biomass in the Bonavista Corridor during the overwintering-spring period measured by acoustic surveys along the shelf edge (with 95% CIs).

void of spawning fish in 1995 and for several years after that (Lilly et al. 2004; also see Fig. 8*b*). Of note, southerly spawning in northern cod in the early 1990s likely resulted in more southerly distributions of juveniles (deYoung and Rose 1993), which could have extended not only into Trinity Bay but to the south coast of Newfoundland and even to the Flemish Cap. (The 1990 and 1992 year classes became relatively strong spawning year classes in all of these areas

Table 2. Tests of genetic homogeneity among samples of Atlantic cod collected from Bonavista Corridor (BVC), Smith Sound (SS), Halibut Channel (HAL), and Hawke Channel (HWK).

Sample 1	Sample 2	*Gmo1*	*Gmo8*	*Gmo19*	*Gmo34*	*Gmo35*	*Gmo36*
SS	BVC	0.0156	0.7799	0.0482	0.0412	0.2378	0.5177
SS	HAL	0.2183	0.1588	0.1599	0.1924	0.2457	0.4095
SS	HWK	0.5530	0.1128	0.0340	0.0040	0.8525	0.2422
BVC	HAL	0.5825	0.2775	0.3213	0.0972	0.5831	0.6402
BVC	HWK	0.0051	0.0483	0.1115	0.0146	0.2084	0.0442
HAL	HWK	0.0196	0.0596	0.1257	0.0014	0.1029	0.1640

Note: *P* values are shown for tests of each locus. Genetic homogeneity between samples is rejected when $P < 0.008$.

Table 3. Pairwise F_{ST} among different samples of Atlantic cod from Bonavista Corridor (BVC), Smith Sound (SS), Halibut Channel (HAL), and Hawke Channel (HWK).

	SS	BVC	HAL	HWK
SS	0.0000			
BVC	*0.0013* (*0.027*)	0.0000		
HAL	0.00084 (0.200)	0.0008 (0.19)	0.0000	
HWK	*0.0044* (*<0.001*)	*0.0037* (*0.018*)	*0.0076* (*0.009*)	0.0000

Note: Italic data represent statistically significant differences, with *P* values in parentheses.

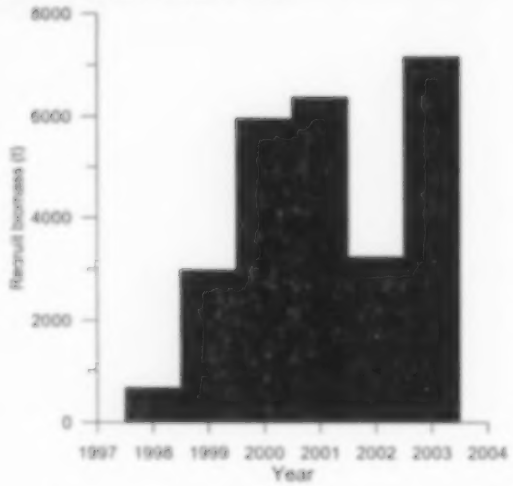
but, somewhat ironically, not in the Bonavista Corridor nor to the north in Hawke Channel (Fig. 4; Gonzalez-Troncoso and Fernandez 2009 for the Flemish Cap).) A similar but reverse situation was reported after a series of warm winters in the North Sea during which the drift of cod eggs and larvae was shifted northward, as were following juvenile distributions (Rindorf and Lewy 2006). These authors emphasized that while drift (retention) had caused the initial distribution shift of early life stages, it was homing that stabilized adult distributions. For the northern cod, a more southerly drift of early life stages seems likely in the cold years of the early 1990s, with northward movements from south coast spawning against the strong southward flowing coastal current far less so (see Pepin and Helbig 1997).

Another possibility related to southern early life stage drift is that immigration to Trinity Bay occurred not during the early life stages, but as migrating subadults (next year first time spawners) from further south. This is particularly applicable to the 1990 year class, which is not well represented in the tagging data from Trinity Bay prior to 1995 and also to the 1992 year class. Tagging data also indicate that migration of subadults and adults from the south coast has occurred in later years, with little indication that the fish returned (Brattey et al. 1999; Lawson and Rose 2000). Such movements become prominent at age 5 according to those tagging data, which would be consistent with the arrival of 1990 year class fish in Trinity Bay in 1995. There is also a more complex hypothesis about the linkages among these groups that will be addressed later in the paper.

Are the Smith Sound cod an isolated genetic population?

A crucial test of any putative linkages between the Smith Sound and adjacent cod groups is their genetic similarity. Ideally, the genetics of fish collected when the aggregation was first detected would have been compared with those of potential offshore parent populations. Ruzzante et al. (1997) reported on cod from Smith Sound collected in 1995, presumably some at least from the founder year classes, but had no samples from any putative parent stock in 1990. Moreover, the 1990 year class did not survive in the Bonavista Corridor, so no such comparisons were possible. The present samples have better defined geographic origins, but only a small percentage of the 791 fish could have been present at the genesis of the large aggregation observed in 1995. Hence our analyses pertain to a time frame 6–7 years after the founding of the Smith Sound aggregation. For these data, pairwise tests of genetic homogeneity indicated that the sample from Hawke Channel was distinct from those from Smith Sound, Halibut Channel, and the Bonavista Corridor (Table 2), which is consistent with previous genetic studies (e.g., Ruzzante et al. 1997, 2001; Beacham et al. 2002). All of these results suggest that Hawke Channel cod are isolated during spawning from Smith Sound cod. Tests of genetic homogeneity were not rejected, however, among the samples from Smith Sound, Halibut Channel, and Bonavista Corridor, suggesting putative demographic linkages within these southern groups (Table 2). Pairwise F_{ST} also showed a significant genetic differentiation between Hawke Channel and all other groups and a small difference between Smith Sound and the Bonavista Corridor (Table 3). Of note, no statistically significant differentiation was indicated between Halibut Channel and Bonavista Corridor or between Halibut Channel and Smith Sound (what we will call the southern complex). To explore the possibility of origin of individual fish without presupposing an origin based on area sampled, the program STRUCTURE (Pritchard et al. 2000) was used to test for groupings based on individual genotypes; this analysis did not detect any significant groupings.

Fig. 9. Estimates of recruited 3- and 4-year-old cod from 1998 to 2003 in Smith Sound, from acoustic survey with age breakdown from trawl samples (see Fig. 6 for *n*).



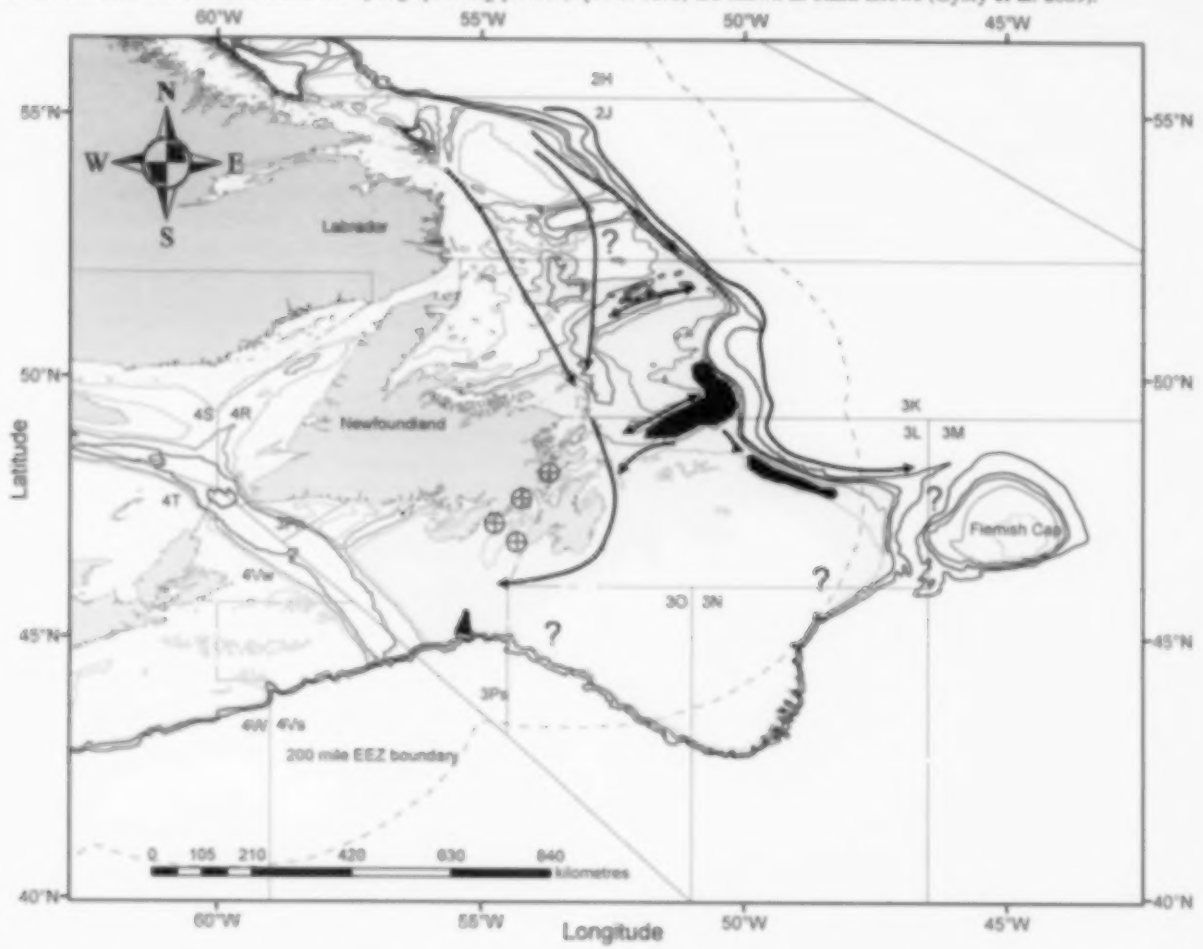
The difference in the present data between Hawke Channel and the southern groups was expected based on their geographic separation and the earlier studies (Ruzzante et al. 1996, 1999; Beacham et al. 2002). However, the lack of differentiation between Smith Sound and the Halibut Channel and between Halibut Channel and the Bonavista Corridor requires more explanation. It is possible that Bonavista Corridor spawning contributed to relatively strong recruitment of fish to both Smith Sound and Halibut Channel in 1989, 1990, and 1992 (and even to the Flemish Cap). Mixing of resident Smith Sound fish with immigrants, bottleneck effects from colonization, and postmigration demographic effects could account for the small difference suggested between Smith Sound and Bonavista Corridor fish. The genetic evidence suggests that the southern groups have had extensive intermixing in the past, and there is little evidence to support the hypothesis that any of the groups studied are isolated and separate genetic populations. More likely, they form components of a metapopulation. Moreover, the weight of the full genetic evidence, taking into account the sampling limitations of previous and present studies, is fully consistent with a remote population contributing to the rapid buildup of mature cod in Smith Sound in the mid-1990s, although it does not unequivocally indicate a source site or the life stage at which the buildup was initiated.

Whither the fish?

After initial increases in biomass in the late 1990s, the Smith Sound overwintering biomass has declined from a maximum of approximately 26 000 t in the early 2000s to a few thousand tonnes in 2009 (Figs. 2*c*, 8*a*). It is unlikely that fishing can account for this decline. Under an assumption that the population growth rate observed from 1995 to 2001 (0.18) would be sustained thereafter, the stock would have grown to about 90 000 t by 2009, accounting for the removals by fishing. Given that population growth may have declined below 0.18, and removals may be underestimated, projections were run with modifications to those parameters, but no reasonable values gave a projection that fit the observed decline. For example, doubling the removals and halving the growth did not reduce the population as observed (Fig. 8*a*). The decline could be tracked, however, under assumptions of zero to slightly negative growth in biomass after 2001, with fishing removals from all of NAFO Divisions 3KL applied to Smith Sound (the extreme case to be considered). The growth assumptions appear to be untenable, however, implying zero or declining individual growth, which is not supported by data (Fig. 7), zero recruitment, also not supported by data (Fig. 9), or increased natural mortality as has been observed offshore (Fudge and Rose 2008), for which there is no evidence in Smith Sound (see data in Rose 2003). Of note, popular reports of increased natural mortality in the winter of 2003 and implications that this group had been decimated were grossly exaggerated (Hoag 2003); in fact, likely 4%–5% of the overwintering Smith Sound fish died (about 800 t (from DFO estimates) of the 19 000 t (from acoustic surveys here reported)). An alternative explanation for the decline is emigration. This could account for the lack of fish on the traditional grounds in the spring of 2009 (Fig. 2*c*). Of note, the steep decline after 2006 suggests migration by the maturing 2002 year class. During the same time period, the biomass of cod overwintering in the Bonavista Corridor has increased from about 10 000 t to approximately 100 000 t (Fig. 8*b*) and has been particularly enriched by unexpected increases in the 2002 year class at ages 5 and 6 (Brattey et al. 2009). How such an exodus might occur is an interesting question. Bonavista Corridor fish are now migrating shoreward and to Trinity Bay after spawning (Brattey et al. 2009), likely pursuing capelin as they did historically and in the early 1990s (Rose 1993). It is possible that maturing cod in Smith Sound (e.g., the 2000 and onward year classes) followed these capelin back offshore in the fall. Movement to another inshore area is also possible. We caution, however, that at present any connection between these events should be regarded as hypothetical. Another possibility is that with the decline in the 1990 and 1992 year classes by the early 2000s (by natural mortality and the fisheries opening in 2001), local recruitment has been insufficient to maintain the biomass at the level attained when these year classes were at their peak. This would add strength to the immigration hypothesis for the founder year classes and could have been a contributing factor to the biomass decline, but it is thought unlikely to explain the full decline (Fig. 8*a*). Unfortunately no year class data are available to test this notion after 2003 (the recruiting of the 1999 and 2000 year classes), at which time recruitment had not declined (Fig. 9). We suspect that the source of the recruitment was local spawning, as all early life stages (Knickle and Rose 2010) and all following life stages (G. Rose, unpublished data) have been present in Smith Sound, but we cannot at present unequivocally link these and separate their impacts from those of immigration. Additional studies are being conducted to address this question.

With respect to mechanisms that underpin stock structure, the present results do not allow any clear separation of retention and adult movements, and both are likely to be im-

Fig. 10. Map of putative metapopulation of Atlantic cod off southern Newfoundland. Uncertainty of northern and eastern boundaries are indicated with question marks. Areas where spawning has been recorded (recognized by females having hydrated eggs and (or) stage 1 eggs in the sea) since 1990 are indicated for the offshore in black regions; inshore areas are shown in bullseyes (compilation from Rose 1993, 2003; Rose et al. 2008; Morgan et al. 1997; Lawson and Rose 2000; Bradbury et al. 2000; Brattey et al. 2008*b*; and G.A. Rose, unpublished data). Labrador Current mean tracks for spring spawning period (April to June) are shown as black arrows (Gyory et al. 2009).



portant. If early life stage retention circumscribes the outer stock boundaries of a large southern metapopulation that includes the Bonavista Corridor, the coastal zone including Smith Sound and likely adjacent northeast coast bays, the northern Grand Bank, and some portion of the south coast (Fig. 10), it would be molded primarily by the inshore branch of the Labrador Current. But the structure would not hold without homing to various spawning grounds (Robichaud and Rose 2001; Thorrold et al. 2001), although not necessarily natal homing (at least none was evident to the Bonavista Corridor in the early 1990s). Moreover, if the founder year classes to Smith Sound were not self-recruited, as there is some reason to suspect, natal homing would not be evident, although homing to spawning grounds has been. Group joining and membership upon reaching maturity, which likely involves learning, cannot be ruled out (Rose 1993; McQuinn 1997). At present we cannot distinguish among these possibilities.

Whatever the mechanisms underpinning the stock structures of cod evident in this region, the various components do not appear to be highly isolated, but capable of spatial pulses of productivity (increase in numbers or biomass) driven perhaps by environmental and migration changes. Such dynamics are similar to a "portfolio" effect as suggested for Pacific salmon (Schindler et al. 2010), with the difference that in this case fish can switch sites. It also appears to demonstrate biocomplexity in productivity rather than in life history characteristics, as noted for Skaggerak cod (Olsen et al. 2008), albeit at much larger scales.

Conclusions for stock rebuilding and management

As with many issues in fisheries, there is no unequivocal answer to the questions initially posed in this paper: "whence and whither the Smith Sound cod". Nevertheless,

fisheries management can seldom wait for the perfect measure, and the weight of the evidence suggests that this aggregation quite likely originated from what might be termed a "biocomplex" event involving shifting distributions of fish within a metapopulation and not from isolated local recruitment. For example, early life stages spawned in the Bonavista Corridor very likely seeded Trinity Bay and areas to the south, and southern migrants could have founded the Smith Sound aggregation, with Smith Sound cod now reinvigorating the Bonavista Corridor, coming full circle. At the very least this possibility, or components of it, cannot be ruled out with the available data. Perhaps the strongest evidence that this hypothesis may be correct is that after a decade of reaggregation each winter in exactly the same place, with spawning following in spring, the Smith Sound cod appear to be disaggregating, presumably to adjacent regions.

If the Smith Sound cod comprise part of such a metapopulation, there are several implications for management. The first is that stock dynamics should not be assumed to be spatially static at contemporary time scales. Cod distributions are clearly not static at longer time scales (Bigg et al. 2008), but rapid changes may not be atypical, particularly as related to ocean climate change (Nye et al. 2009; Ruppert et al. 2009) or variability (Vilhjalmsson 1997). Stock assessments and management will have to take such phenomena into account. On a related issue, we caution against any interpretation of our results and those of others (e.g., Smedbol and Wroblewski 2002) as being counter to the importance of fine-scale genetic and spatial structure (e.g., Beacham et al. 2002; Bradbury et al. 2008; Hauser and Carvalho 2008). To the contrary, although we may differ on the mechanisms underpinning stock structure, we interpret the present results as reinforcing the importance of spatial diversity, in particular if these spatial cod groups are subject to a portfolio of productivity. In cases where stock rebuilding is a management goal, it is of prime importance to allow extant and seemingly isolated and locally productive groups to grow. Increasing spatial heterogeneity and local density should lead to a greater potential for metapopulation components to undertake range expansion or outright emigration (Stephenson 1999). To ignore these issues is at best risk-prone and at worst could lead to serial depletion of existing groups. There has been a moratorium on all areas of offshore northern cod within the Canadian Exclusive Economic Zone since 1992 and only small annual harvests from the inshore zone (recently 3000–5000 t), but more substantial fisheries in the southern zone (NAFO Subdivision 3Ps) have taken place over the past decade, and the adjacent Flemish Cap cod stock is staged to be fished in 2010. These fisheries have in all likelihood prolonged the period of little overall stock growth (Shelton et al. 2006) and may have precipitated additional declines.

In closing, although the conclusions we have drawn apply in particular to the Newfoundland cod, we believe they may have applicability to other species and regions. Of note, these results suggest that stock structure in marine fishes may be highly complex and subject to change at contemporary time scales. As a general rule, we recommend that interpretations of stock structure consider all available sources of information (historical, demographic, and genetic) and that it be understood that climate change will likely disrupt any perceived stability.

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PERSPECTIVE / PERSPECTIVE

Blowin' in the wind: reciprocal airborne carbon fluxes between lakes and land [1]

M. Jake Vander Zanden and Claudio Gratton

Abstract: Ecologists are increasingly interested in how disjunct habitats are connected through the cross-habitat movement of matter, prey, nutrients, and detritus and the implications for recipient systems. The study of lake ecosystems has been dominated by the study of pelagic (open-water) production and processes, though there is growing awareness of the role of terrestrial inputs and benthic trophic pathways. Here, we review the phenomena of airborne fluxes to and from lakes. We assemble published data on terrestrial particulate organic carbon (TPOC) deposition to lakes, insect production, and insect emergence and use these data to simulate how airborne lake-to-land and land-to-lake carbon flux is expected to scale with ecosystem size, while taking into account among-lake variability in emergence and TPOC deposition. Emergent insect flux to land increases as a function of lake size, while TPOC deposition to lakes decreases as a function of lake size. TPOC deposition exceeds insect emergence in small lakes, while in large lakes, insect emergence exceeds TPOC deposition. We present a general framework for considering directional fluxes across habitat boundaries. Furthermore, our results highlight the overarching role of ecosystem geometry in determining insect emergence, airborne carbon deposition, and net carbon flux between adjacent ecosystems.

Résumé : Les écologistes sont de plus en plus intéressés à savoir comment les habitats séparés sont reliés par des mouvements croisés de la matière, des proies, des nutriments et du détritus entre les habitats et aussi à en connaître les conséquences sur les systèmes récipiendaires. L'étude des écosystèmes lacustres a été dominée par la détermination de la production et des processus pélagiques (en eau libre), bien qu'il y ait une perception croissante du rôle des apports terrestres et des voies trophiques benthiques. Nous faisons une rétrospective du phénomène des flux atmosphériques provenant des lacs et y aboutissant. Nous rassemblons les données publiées sur les précipitations de carbone organique particulaire terrestre (TPOC) dans les lacs, sur la production des insectes et sur l'émergence des insectes et nous utilisons ces données pour simuler comment les flux atmosphériques de carbone du lac vers le milieu terrestre et du milieu terrestre vers le lac sont fonction de la taille de l'écosystème, tout en tenant compte de la variabilité de l'émergence et des précipitations de TPOC entre les lacs. Le flux d'insectes émergés vers le milieu terrestre augmente en fonction de la taille du lac, alors que les précipitations de TPOC dans les lacs diminuent en fonction de la taille du lac. Les précipitations de TPOC sont supérieures à l'émergence des insectes dans les petits lacs, alors que, dans les grands lacs, l'émergence des insectes dépasse les précipitations de TPOC. Nous présentons un cadre général pour l'étude des flux directionnels à travers les frontières des habitats. De plus, nos résultats soulignent le rôle prédominant de la géométrie de l'écosystème dans la détermination de l'émergence des insectes, des précipitations atmosphériques de carbone et du flux net de carbone entre les écosystèmes adjacents.

[Traduit par la Rédaction]

Introduction

> A paradigm forged on the belief that what matters happens in the open-water pelagic just had to change.

The above quote from Reynolds (2008, p. 527) adds to a growing chorus of challenges to the long-standing "pelagiocentric" paradigm of lake ecosystem function (Schindler and Scheuerell 2002; Vadeboncoeur et al. 2002; Cole et al. 2006). This expanding view parallels the more general acknowledgement that disjunct habitats can be coupled via food web linkages and that these cross-habitat couplings can have important implications for recipient food webs (Polis et al. 1997, 2004). Habitats can be linked through the movement of predators, prey, detritus, or nutrients — such linkages many be ultimately driven by migration, gravity,

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M.J. Vander Zanden.[2] Center for Limnology, University of Wisconsin – Madison, 680 N. Park Street, Madison, WI 53706, USA.
C. Gratton. Department of Entomology, University of Wisconsin – Madison, Madison, WI 53706, USA.

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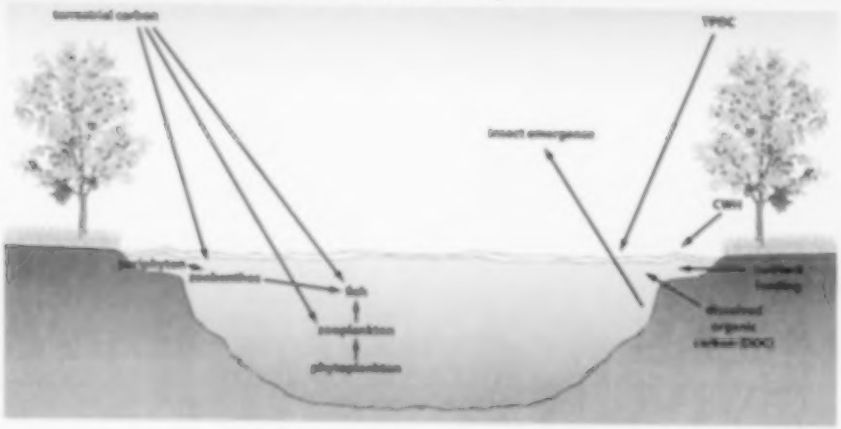
[2]Corresponding author (e-mail: mjvanderzand@wisc.edu).

Fig. 1. A generalized north-temperate lake. Left side: some of the major carbon flow pathways. Right side: cross-habitat linkages considered in this paper. TPOC, terrestrial particulate organic carbon; CWH, course woody habitat.



currents of water and air, or human activities. These cross-habitat linkages can have important implications for the dynamics of recipient systems. Notable examples include marine inputs subsidizing desert island food webs off the coast of Baja California, Mexico (Polis and Hurd 1995, 1996) and the reciprocal cross-habitat food web linkages involving small streams and their surrounding riparian habitat (Nakano et al. 1999; Nakano and Murakami 2001).

In contrast with many terrestrial continental ecosystems, lakes have often been envisioned as habitat islands in a sea of land (Forbes 1887). Conveniently, they have discrete borders, and for the purpose of investigations, can be viewed as "closed" ecosystems. This feature makes them appealing systems for ecological and biogeochemical study, and several important ideas in the history of ecology derive from the study of lakes as habitat islands (Lindeman 1942; Hutchinson 1959; Carpenter et al. 1985). At the organismal level, it is true that many of the dominant and well-studied lake inhabitants such as fish are confined to lakes for the entirety of their lives. On the other hand, it is widely recognized that lakes are subject to a directional flux of sediments and nutrients from land (Sharpley et al. 1994; Carpenter et al. 1998; Smith and Schindler 2009). Human activities such as agriculture and urbanization greatly exacerbate nutrient inputs to lakes, with major environmental and economic consequences (Sharpley et al. 1994; Carpenter et al. 1998). In addition, many lakes are net heterotrophic systems, necessitating carbon inputs from outside the system (del Georgio and Peters 1994; del Giorgio et al. 1997; Hanson et al. 2003).

The unidirectional nature of the paradigm of lake–land linkages contrasts with the situation for stream ecosystems. Early research and conceptual models in stream ecology highlighted the dominant role of allochthonous inputs as a driver of stream ecosystem carbon and energy budgets (Fisher and Likens 1973; Hynes 1975; Vannote et al. 1980). Upstream–downstream as well as lateral (i.e., main channel – floodplain) habitat linkages have been challenging to study, but are well-integrated into stream ecologists' worldview (Vannote et al. 1980; Junk et al. 1989; Malmqvist 2002), such that streams are generally viewed as a tightly integrated part of the broader watershed ecosystem (Hynes 1975; Allan 1995; Malmqvist 2002). One contributing factor is that lotic systems tend to be more compact in spatial extent relative to lentic systems: the land–water ecotone for streams is visually obvious, and the fact that terrestrial vegetation often overhangs streams is a further reminder of the potential for connectivity between streams and the surrounding terrestrial landscape. In the last decade, a body of experimental research has elucidated the interconnectivity and interdependence of stream and terrestrial food webs (Nakano et al. 1999; Nakano and Murakami 2001; Baxter et al. 2005).

Perhaps it is not surprising that our understanding of the relevant processes linking aquatic and terrestrial habitats (i.e., secondary production, aquatic insect emergence) derives primarily from research on stream ecosystems. In this paper we address airborne cross-habitat linkages involving lake ecosystems, with emphasis on integrating our current understanding of land-to-lake and lake-to-land linkages. We use carbon as a currency for the ecosystem because it is the most commonly used unit for studies of ecosystem production and energy flow, though ecosystem fluxes could also be represented using mass, energy, phosphorus, or nitrogen.

We start by providing a thumbnail sketch of carbon flows in a generalized north-temperate lake ecosystem (Fig. 1, left side). At the base of the food web, in-lake (autochthonous) primary production occurs in both pelagic (phytoplankton) and benthic (periphyton) habitats. Lakes also receive allochthonous carbon (in various forms) from the watershed, providing an additional potential carbon source to the base of the food web. Dominant primary consumer groups are zooplankton and zoobenthos — these groups rely on a variable mix of terrestrial, benthic, and pelagic carbon sources. There may be additional invertebrate trophic levels (invertebrate predators) within both zooplankton and zoobenthos. These groups provide food for invertivorous fishes, which in turn may be fed upon by piscivorous fishes. The central foci of this paper are nonmicrobial food web linkages that support higher trophic levels such as fishes. Lake-wide carbon cycling and microbial processes are beyond the scope of this paper and have been addressed elsewhere (del Georgio and Peters 1994; del Giorgio et al. 1997; Hanson et al. 2003).

Land-to-lake linkages

Lakes, by definition, tend to occupy low spots or depressions on the landscape. As a result, they are subject to the downhill, gravity-driven delivery of terrestrial-derived materials: organic and inorganic matter, organisms, and nutrients (Likens et al. 1970). Pathways for this land-to-lake transport include stream inflows, groundwater, and wet or dry airborne deposition (Fig. 1, right side). We review these land-to-lake linkages below, emphasizing the role of airborne pathways.

Nutrient loading to lakes

By far the most well-documented linkage between lakes and land is the input of terrestrial-derived nutrients into lakes (Hasler 1975; Sharpley et al. 1994; Carpenter et al. 1998). A large body of research has since linked excess loading of phosphorus from agricultural, municipal, and industrial sources to degradation of water quality and excess algal blooms (Schindler 1974, 1977), prompting efforts to reduce point sources and nonpoint source nutrient runoff to aquatic ecosystems. Nitrogen and phosphorus also enter lakes via atmospheric pathways. In agricultural or urban watersheds, the atmosphere is likely to be secondary in importance to that of nutrient runoff. Most atmospheric nitrogen is anthropogenic in origin and derives from fossil fuel combustion and volatilization from agricultural lands (Carpenter et al. 1998). Atmospheric deposition is the main source of nitrogen as well as an important source of phosphorus to Lake Tahoe and other alpine lakes (Goldman 1961; Jassby et al. 1994). Cole et al. (1990) found that atmospheric fallout was a significant source of phosphorus to Mirror Lake, greatly exceeding stream-derived phosphorus loading. Atmospheric nitrogen deposition is closely linked to watershed export of nitrogen and the resulting coastal zone eutrophication (Howarth et al. 1996; Jaworski et al. 1997). These examples highlight the fact that atmospheric nutrient pathways can have important implications for aquatic systems and water quality.

Terrestrial dissolved organic carbon (TDOC) inputs to lakes

Terrestrial organic carbon enters lakes in either particulate or dissolved form. Dissolved organic carbon (DOC) is a component of rainwater (Willey et al. 2000), though most DOC loading to lakes is received directly from the catchment and is not atmospheric in origin. Catchment-derived DOC flux to lakes is driven by factor such as water residence time and the occurrence and position of wetlands in the watersheds relative to lakes (Dillon and Molot 1997*a*, 1997*b*; O'Connor et al. 2009).

DOC plays a remarkably important role in structuring lake ecosystems (Wetzel 2001). It strongly influences lake transparency and mixing depth (Fee et al. 1996), bioaccumulation of methylmercury (Watras et al. 1998), attenuation of UV radiation (Morris et al. 1995), and is a key substrate for microbial production. Though terrestrial DOC fuels pelagic bacterial respiration in lakes, it is generally not efficiently passed to higher trophic levels (Cole et al. 2002, 2006).

Terrestrial particulate organic carbon (TPOC) inputs to lakes

TPOC in lakes can be operationally defined as material larger than 0.45 μm in diameter. TPOC inputs to lakes can be of diverse origins and include leaves, twigs, insects and insect parts, pollen, and airborne dust. Studies of TPOC inputs to lakes have focused primarily on litterfall and atmospheric deposition, though streams and direct surface runoff are also potential sources of TPOC to lakes.

The relatively few empirical studies of TPOC deposition have been limited with regards to particle size or season. Studies have either quantified leaf (coarse TPOC) inputs during the fall season (Gasith and Hasler 1976; France and Peters 1995) or measured fine organic matter inputs during summer (Cole et al. 1990; Preston et al. 2008). TPOC loading rates are highly variable among lakes. Overall rates of litterfall inputs to lakes are very much influenced by terrestrial vegetation type and forest cover (Rau 1976; France and Peters 1995). Furthermore, within lakes the magnitude and nature of TPOC inputs are highly variable in space and time, depending on factors such as local vegetation type, location relative to the dominant wind direction, and major weather events (Preston 2009). For leaf litterfall inputs, there is generally a rapid decline as a function of distance from shore, with most leaf litter inputs to lakes occurring within 10 m of shore (Gasith and Hasler 1976; France and Peters 1995). Inputs of fine POC to lakes are also elevated adjacent to shore, though there is notable deposition extending substantial distances out into the lake (Preston et al. 2008; Preston 2009).

Insects and coarse woody habitat as TPOC

A notable fraction of airborne organic matter inputs to lakes consists of terrestrial arthropods and arthropod fragments. Though terrestrial insects are rarely dominant in lentic fish diets, they are commonly present (Hunt 1975; Weidel et al. 2008). Mehner et al. (2005) reported that the diet of bleak (*Alburnus alburnus*) was dominated by terrestrial insects (~84%), with potential impacts on the phosphorus budget of the lake. Rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) from Castle Lake, California, consumed >30% terrestrial prey (Vander Zanden et al. 2006). Even siscowets, the deepwater form of lake trout (*Salvelinus namaycush*) from Lake Superior, were found to consume terrestrial insects (Sitar et al. 2008). Francis and Schindler (2009) found that fish consumption of terrestrial arthropod prey declined with increasing lakeshore development. They also found a positive effect of terrestrial prey on salmonid consumption rates, indicating the functional importance of this poorly appreciated cross-habitat link.

Terrestrial arthropods can affect lake ecosystems, aside from their role as fish food. In the summer of 1979, millions of ants were blown into Castle Lake, California, on two occasions, causing notable pulses in water column ammonium concentrations (Carlton and Goldman 1984). In several areas of eastern North America, emergences of 17-year cicada broods produced large inputs of organic matter in the form of cicada carcasses to small aquatic ecosystems (Nowlin et al. 2007; Pray et al. 2009). Although these are dramatic examples, they highlight the potential role of terrestrial arthropods in linking terrestrial and aquatic systems.

Finally, trees and branches from riparian vegetation fall into lakes and can be viewed as particularly large packages of TPOC. Because wood decomposition rates are slow,

fallen trees provide course woody habitat (CWH) to aquatic systems (Schindler and Scheuerell 2002; Francis and Schindler 2006). Recent work has demonstrated a strong negative relationship between CWH and housing density, stemming from the fact that humans tend to remove trees and CWH from lake shorelines (Christensen et al. 1996). CWH is an important refuge habitat for invertebrates and fish and plays an important role in stabilizing predator–prey dynamics (Sass et al. 2006). Schindler et al. (2000) reported a positive relationship between CWH density and fish growth rates.

Implications of terrestrial carbon inputs to lakes

Recent studies have assessed the role of terrestrial-derived organic carbon to lake metabolism and the production of higher trophic levels. Dystrophic and unproductive lakes are well-documented to be net heterotrophic, meaning that bacterial respiration is greater than phytoplankton production (del Georgio and Peters 1994; del Giorgio et al. 1997; Hanson et al. 2003). This net heterotrophy requires an external carbon source, and the role of allochthonous DOC inputs in fueling bacterial respiration is widely accepted (Hanson et al. 2003).

Studies have assessed the influence of terrestrial-derived carbon on the classical (nonmicrobial) food web. Graham et al. (2006) found that addition of conifer pollen to experimental mesocosms subsidized nutrient levels, producing increased phytoplankton, periphyton, and zooplankton biomass. Terrestrial leaf litter partially supports benthic macroinvertebrate production in lakes (Rau 1976; Solomon et al. 2008). Stable isotope studies suggest that zooplankton rely on a mix of autochthonous and allochthonous carbon (Karlsson et al. 2003). Differentiating autochthonous and allochthonous carbon pathways using stable carbon isotopes can be confounded when sources have overlapping $\delta^{13}C$ values. This problem has been overcome through whole-lake ^{13}C addition experiments. These experiments indicate that in-lake POC and zooplankton in small north-temperate lakes were up to 50% terrestrial in origin (Pace et al. 2004). Terrestrial carbon inputs to aquatic food webs may ultimately be derived from one of several pathways: TDOC, TPOC, or terrestrial prey, defined as terrestrial insects or vertebrates that intentionally or accidentally enter lakes and can be consumed by fish. Cole et al. (2006) reported that TDOC dominated bacterial respiration, but was a minor contributor to higher trophic levels of either benthic or pelagic food webs. In contrast, TPOC inputs were readily incorporated into pelagic and benthic invertebrates, while terrestrial prey items were a major carbon source for certain fish species.

Terrestrial carbon inputs can also affect lentic food webs in reservoir ecosystems containing gizzard shad (*Dorosoma cepedianum*) in the southeastern United States. Gizzard shad exert dramatic direct and indirect food web impacts on phytoplankton, zooplankton, and planktivorous and piscivorous fishes. They can be very abundant and have such impacts because they are omnivorous and consume large amounts of terrestrial detritus (Stein et al. 1995; Vanni et al. 2005). Detritus is abundant in many reservoir systems and subsidizes gizzard shad populations, thereby effectively decoupling them from in situ prey such as zooplankton (Stein et al. 1995; Vanni et al. 2005). The consumption of terrestrial resources allows gizzard shad to play a keystone role in structuring many reservoir ecosystems.

In summary, there are diverse pathways by which organic matter and nutrients of terrestrial origin can be transported to lake ecosystems. These inputs can have important implications for the structure and function of these ecosystems. Inputs of terrestrial-derived nutrients are the major driver of lake eutrophication, an environmental problem of global concern. In addition, terrestrial-derived carbon affects a wide range of lake ecosystem functions: water transparency, UV penetration, water chemistry, and ecosystem metabolism, in addition to partially supporting the production of higher trophic levels.

Lake-to-land linkages

Our understanding of the linkages between lakes and the surrounding landscape has been primarily focused on land-to-lake linkages (Schindler and Scheuerell 2002). Yet a wide range of taxa, including birds, mammals, reptiles, amphibians, fish, and insects, have behaviors and life histories that can couple aquatic and terrestrial habitats, often in complex and reciprocal ways. Perhaps the most potent vector for lake-to-land linkages is aquatic insects. Aquatic insects have complex life histories — typically there is a larval aquatic stage lasting anywhere from several weeks to several years, followed by metamorphosis into a terrestrial adult stage. Only recently have studies examined the role of aquatic insects in linking lakes and surrounding terrestrial habitats and the resultant implications for terrestrial food webs (Finlay and Vredenburg 2007; Gratton et al. 2008; Pope et al. 2009). In a study of 16 lakes from the Trinity Alps Wilderness, California, Pope et al. (2009) found that aquatic insect emergence increased in response to removal of non-native trout and that trout density determined insect emergence from alpine lakes. Along these same lines, Finlay and Vredenburg (2007) found that lakes in the Sierra Nevada mountains of California with introduced trout populations had dramatically reduced (~20×) levels of aquatic insect emergence. These aquatic insects were important prey for terrestrial consumers, particularly mountain yellow-legged frog (*Rana muscosa*), which were ten times more abundant in the absence of non-native trout. This study demonstrated how the introduction of trout severed the food web link between lakes and the surrounding "terrestrial" consumers (adult frogs), leading to declines in native amphibians and potentially their terrestrial predators as well.

Gratton et al. (2008) quantified aquatic insect (midge) infall into terrestrial habitats adjacent to Icelandic lakes. Rates of midge infall to the terrestrial ecosystem were highly variable among lakes, declined with distance from the lakeshore, and at some lakes, were likely sufficient to produce an important fertilizing effect on terrestrial vegetation. Carbon isotope ratios also revealed that near lakes with large midge populations, many terrestrial consumers were highly dependent upon midge-derived trophic pathways (Gratton et al. 2008). A broad literature review of aquatic insect dispersal from aquatic systems revealed that insect deposition to terrestrial ecosystems shows a negative exponential decline as a function of distance from shore (Gratton and Vander Zanden 2009), indicating that this linkage is spatially constrained.

Knight et al. (2005) found that the presence of predatory fish in Florida ponds reduced local abundance of larval and adult dragonflies. Dragonflies themselves prey on other insects as larvae and adults. The reduction in adult dragonflies in the presence of fish resulted in nearby terrestrial plants receiving more visits from pollinators, thereby increasing plant reproductive output. Amphibians can also couple aquatic and terrestrial systems. When salamander larvae metamorphose into terrestrial adults, they transfer substantial amounts of pond-derived production to terrestrial habitats, while oviposition by females transfers terrestrial-derived production back to aquatic systems, highlighting the potential for lentic and terrestrial habitats to be coupled in a reciprocal manner (Regester et al. 2006, 2008).

Carbon flux across the lake–land ecotone

In the above two sections, we surveyed the literature on cross-habitat linkages between lakes and the surrounding terrestrial landscape, highlighting examples of linkages occurring in both directions (i.e., lake-to-land and land-to-lake). Though individual studies have assessed fluxes across this ecotone, an important remaining task is to identify and quantify the factors that modulate the individual and net fluxes in both directions. To do so, we build upon the model of Gratton and Vander Zanden (2009), which considered aquatic productivity and ecosystem geometry as controlling variables modulating the export of aquatic production to land. Here, we extend this model to also include TPOC deposition to lakes and how lake-to-land, land-to-lake, and net airborne carbon flux scale with lake size (variables and symbols used in our calculations are indicated in Table 1). First, we provide a brief overview of three key factors that affect airborne carbon fluxes to and from lakes: aquatic productivity, TPOC deposition, and ecosystem geometry. Following this, we develop the two components of the model (lake-to-land and land-to-lake) and use numeric simulations and the limited existing empirical data to attempt to quantitatively compare the cross-habitat fluxes.

Aquatic production

The magnitude of aquatic insect flux from lakes is constrained by several factors, the most fundamental being aquatic secondary production. Production is an important and potentially unifying concept in ecology (Odum 1968) and represents the rate of generation of new biomass over a given time period. It can be estimated at multiple ecological levels, ranging from populations to whole ecosystems, and provides a common currency for characterizing the flow of carbon through food webs and ecosystems (Lindeman 1942; Odum 1968; Waters 1977).

The basic method for estimating secondary production for a benthic invertebrate population dates to Boysen-Jensen (1919). Since then there have been methodological advances, though the basic idea remains the same and involves tracking the growth and mortality of a cohort of individuals through time — production is calculated as the area under the curve of a plot of number of individuals versus mean mass (the method is also referred to as the Allen curve, increment summation, instantaneous growth, or the cohort methods; Waters 1977). In reality, many populations do not show clear cohorts because of overlapping generations. In such cases, one can assume that the size-frequency distribution of individuals collected throughout the year reflects that of the "average" cohort, thereby allowing production to be approximated (called the size-frequency method; Waters 1977). Investigators have found that production per unit biomass (P/B, turnover rate) does not vary widely for a given taxon. Thus investigators sometimes estimate production as the product of mean annual biomass (estimated from field sampling) and literature-derived P/B values. Studies have also found that P/B varies as a function of body size and temperature, thus allowing for allometric-based empirical methods for estimating production from field biomass data (Banse and Mosher 1980; Plante and Downing 1989). This is particularly useful when scaling production estimates up to the community or ecosystem scale.

Aquatic secondary production is the most widely available indicator of potential insect emergence in lakes. Of course, only a fraction of invertebrate secondary production consists of aquatic insects, which are the taxonomic group that emerges from lakes. Furthermore, only a fraction of the aquatic insect production from a lake emerges and thus potentially links aquatic and terrestrial systems. Finally, some fraction of emergent insects ultimately return to the lake in the form of insect carcasses and either sink, decompose, wash to shore, or are consumed. Though we view aquatic secondary production as an important factor regulating aquatic insect flux from lakes to land, the combination of factors noted above ultimately determine insect emergence and the potential flux to land.

TPOC deposition

The second factor in our generalized model of airborne carbon fluxes to and from lakes is TPOC deposition. As noted above, we define TPOC as terrestrial-derived particles that are deposited upon lake surfaces. For the sake of simplicity, our model ignores airborne DOC in rainwater and non-airborne or non-atmospheric TPOC sources (river inflows and overland deposition). TPOC is diverse in origin and includes pollen, leaf litterfall, insects, and airborne dust. As such, it is difficult to generalize with regard to chemical composition, lability, and food quality. Furthermore, rates of TPOC deposition onto lake surfaces are highly variable among lakes, and for individual lakes, TPOC inputs can vary widely in space and through time (Preston et al. 2008; Preston 2009). Terrestrial particle deposition is typically measured using floating collectors distributed on the lake surface, from which total deposition can be approximated. Studies often separate large and small fractions (for example, Preston et al. 2008 considered large to be >153 μm and small as 0.7–153 μm).

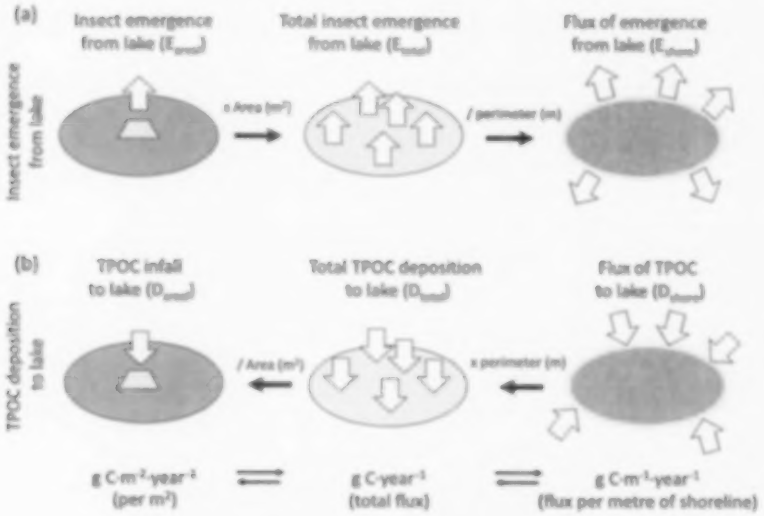
Ecosystem geometry

The third factor mediating airborne aquatic–terrestrial linkages is ecosystem geometry, which refers to both ecosystem size and shape. Lakes can be simplified by reducing their complex geometries into two easily quantifiable attributes: lake perimeter (p) and area (A). Ecosystem geometry is fundamental in mediating the strength of land–lake linkages, primarily because it determines the perimeter to area ratio (p/A) (Polis and Hurd 1996). Small lakes are dominated by

Table 1. Variables used in this study.

Variable	Symbol	Unit	Equation
Shoreline development factor	D_L	—	$p/[2(\pi A)^{1/2}]$
Lake perimeter	p	m	—
Lake area	A	m^2	—
Lake radius	r	m	$(A/\pi)^{1/2}$
Benthic secondary production	P_b	g C·m^{-2}·year^{-1}	—
Fraction of benthic secondary production consisting of insects	f_i	(Fraction)	—
Benthic insect production	P_i	g C·m^{-2}·year^{-1}	$P_b \times f_i$
Emergence			
Fraction of benthic insect production that emerges from the lake	f_e	(Fraction)	—
Insect emergence	E_{areal}	g C·m^{-2}·year^{-1}	$P_i \times f_e$
Total flux, aquatic to terrestrial	E_{total}	g C·year^{-1}	$E_{areal} \times A$
Emergent insect flux: per metre of shore	E_{shore}	g C·m^{-1}·year^{-1}	E_{total}/p
Deposition			
Shoreline deposition: terrestrial deposition per metre of shoreline	D_{shore}	g C·m^{-1}·year^{-1}	—
Total flux, terrestrial to aquatic	D_{total}	g C·year^{-1}	$D_{shore} \times p$
Terrestrial deposition: per square metre of lake	D_{areal}	g C·m^{-2}·year^{-1}	D_{total}/A

Fig. 2. Illustration of our approach for estimating lake-to-land and land-to-lake carbon fluxes. (*a*) Aquatic insect emergence from lakes. Calculations in this study moved from left to right. (*b*) TPOC deposition to lakes. Calculations in this study moved from right to left (see variables listed in Table 1).

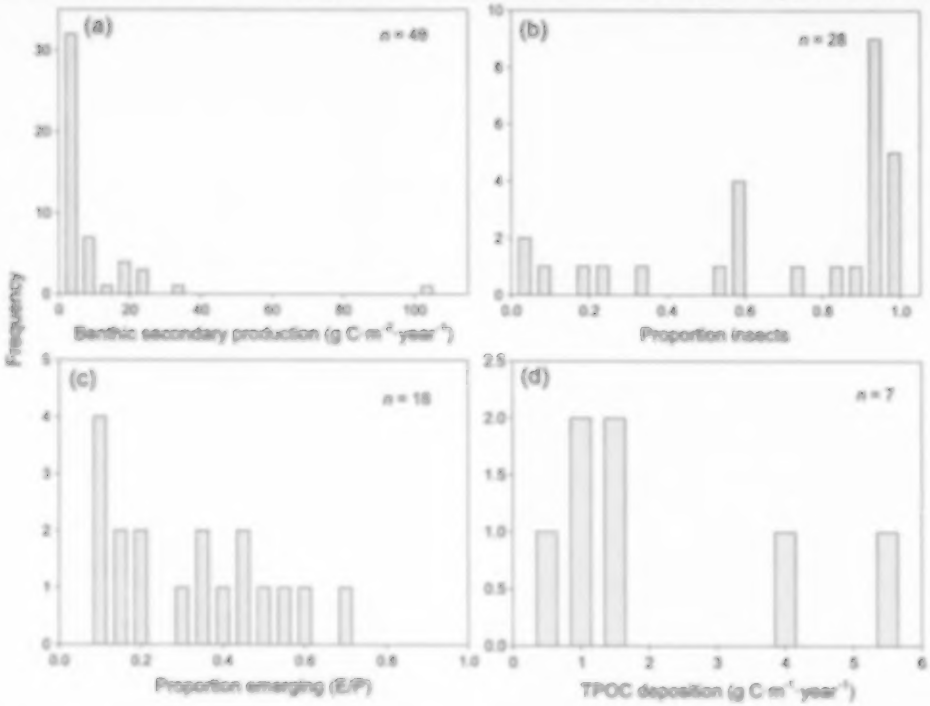

(a)
Insect emergence from lake (E_areal)
Total insect emergence from lake (E_total)
Flux of emergence from lake (E_shore)
Insect emergence from lake
x Area (m²)
/ perimeter (m)
(b)
TPOC infall to lake (D_areal)
Total TPOC deposition to lake (D_total)
Flux of TPOC to lake (D_shore)
TPOC deposition to lake
/ Area (m²)
x perimeter (m)
g C·m⁻²·year⁻¹ (per m²)
g C·year⁻¹ (total flux)
g C·m⁻¹·year⁻¹ (flux per metre of shoreline)

"edge" or shoreline effects (i.e., p/A is relatively large) because the entire lake can be in close proximity to the shoreline, similar to the situation for streams. In contrast, large lakes have less shoreline relative to their surface area (p/A is smaller). In addition, lake shape determines the overall shoreline length for a given lake size. The shoreline development factor ($D_L = p/[2(\pi A)^{1/2}]$, where p is perimeter (m) and A is area (m^2)) is a widely used measure of shoreline complexity in which lake perimeters (p) are compared with the perimeter of an idealized circular lake of similar area. D_L ranges from 1.0 for a perfectly circular lake to >15 for some highly reticulate reservoir systems (Kalff 2002).

Modeling lake-to-land flux

Our approach for examining insect emergence from lakes is based on Gratton and Vander Zanden (2009) (illustrated in Fig. 2*a*). Some fraction (f_i) of lake benthic secondary production (P_b) consists of benthic insects (P_i). Aquatic insects are the sole taxonomic group that emerges from lakes, thus potentially linking aquatic and terrestrial habitats. Some fraction of benthic insect production emerges (f_e) from the lake (E_{areal}). Based on the above, $E_{areal} = P_b \times f_i \times f_e$ (note that P_b, P_i, and E_{areal} are expressed on a per square metre basis; g C·m^{-2}·year^{-1}). Total aquatic insect flux is calculated as the product of insect emergence E_{areal} and lake area A

Fig. 3. Literature-derived data used in our simulations: (*a*) benthic secondary production (P_b), (*b*) proportion of benthic secondary production comprised of insects (f_i), (*c*) fraction of insect production that emerges (f_e), and (*d*) TPOC deposition per unit shoreline (D_{shore}).



(m^2): $E_{total} = E_{areal} \times A$ (g C·year^{-1}). Flux per metre of shoreline (E_{shore}, g C·m^{-1}·year^{-1}) is estimated by dividing total flux by lake perimeter: $E_{shore} = E_{total}/p$. For a circular lake, $A = \pi r^2$ and $p = 2\pi r$, where r is the lake radius. Variation in lake shape can also be accounted for by including the shoreline development factor: $E_{shore} = E_{areal} \times r(2D_L)$ (Gratton and Vander Zanden 2009).

We give the above conceptual framework an empirical footing using a combination of literature-derived and simulated data for the above parameters (Gratton and Vander Zanden 2009). Benthic secondary production and insect emergence data were available for a broad cross-section of lake types from around the world, though there are surprisingly few estimates available. Of the 49 whole-lake benthic secondary production estimates for lakes, only 14 were published after 1980. The majority of the production estimates dated from the International Biological Programme of the 1960s and 1970s. These data were used to simulate potential fluxes of aquatic insects to land in a previous study (Gratton and Vander Zanden 2009).

Values of benthic secondary production (P_b) were highly right-skewed, with a median value of 2.3 g C·m^{-2}·year^{-1} (Fig. 3*a*). On average, insects comprised about 70% of benthic secondary production (f_i = 0.70 ± 0.33, mean ± 1 standard deviation, SD; Fig. 3*b*), while approximately 30% of insect production leaves lakes in the form of insect emergence (f_e = 0.30 ± 0.04, mean ± 1 SD; Fig. 3*c*). Because insect production and emergence were independent of lake area, we used these data to simulate how production and ecosystem size interact to determine the flux of aquatic insects to land (Gratton and Vander Zanden 2009). We randomly selected a value of P_b, f_i, and f_e from our empirical data (n = 49, 28, and 18, respectively) and calculated the product, which is a pseudo-estimate of insect emergence ($E_{areal} = P_b \times f_i \times f_e$; g C·m^{-2}·year^{-1}). This was repeated 5000 times. We then generated 5000 corresponding values of lake area (A, m^2) from a hypothetical lake size distribution with a constant number of lakes in each $\log_{10}$ size class. For each simulated lake, we calculated total emergence ($E_{total} = E_{areal} \times A$; g C·year^{-1}) and insect flux per metre of shoreline ($E_{shore} = E_{total}/p$; g C·m^{-1}·year^{-1}). For simplicity, all lakes in our model were treated as circular.

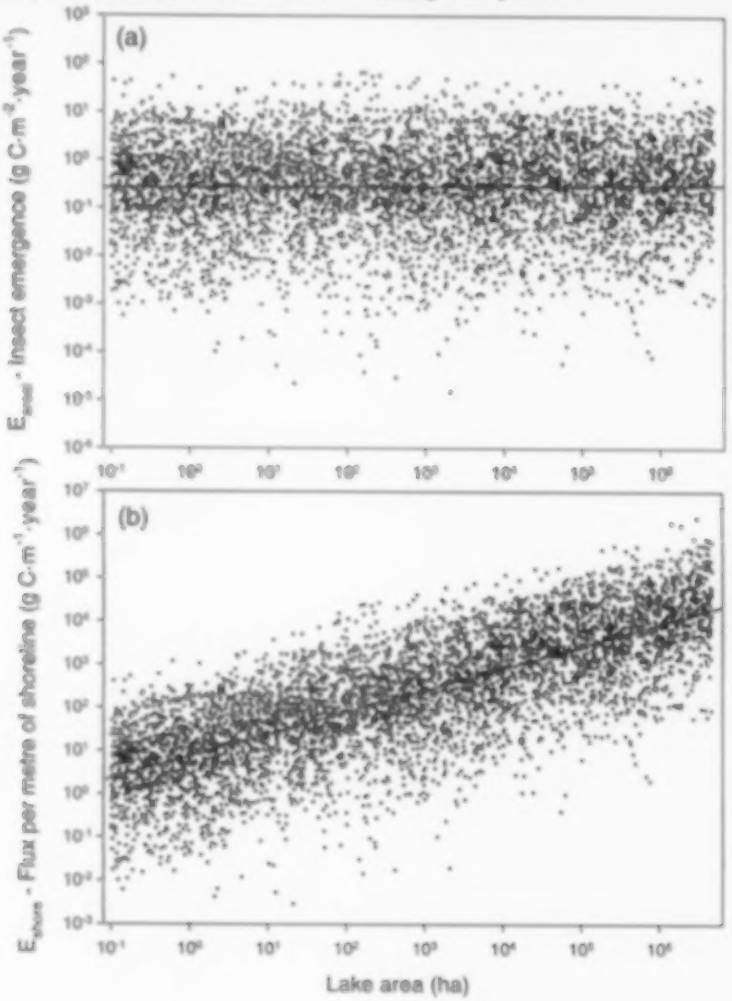
Results: lake-to-land

Our estimates of insect emergence (E_{areal}, g C·m^{-2}·year^{-1}) varied widely among lakes and were independent of lake size (Fig. 4*a*). Expressing emergence as the flux per metre of shoreline (E_{shore}, g C·m^{-1}·year^{-1}) has the effect of incorporating ecosystem geometry, such that E_{shore} increases with lake size (Fig. 4*b*). The residual scatter (Figs. 4*a* and 4*b*) reflects the among-lake variation in secondary production and insect emergence. The positive relationship with lake size is intuitive because the amount of lake surface area per metre of shoreline increases as a linear function of lake radius (and thus area).

Modeling land-to-lake flux

A similar conceptual approach as above was used to estimate terrestrial-derived aerial inputs to lakes, though applied in reverse (Fig. 2*b*, moving right to left). We used empirical

Fig. 4. Scaling of lake-to-land airborne carbon flux with lake size based on 5000 simulated lakes: (*a*) insect emergence on an areal basis: E_{areal}, g C·m^{-2}·year^{-1}; (*b*) emergent insect flux per metre of shoreline: E_{shore}, g C·m^{-1}·year^{-1}.



estimates of airborne carbon deposition, D_{shore}, expressed per metre of shoreline (g C·m^{-1}·year^{-1}, Fig. 2*b*), as presented in Preston et al. (2008). Theoretically, D_{shore} should be independent of the size of the recipient lake, since it is expressed on a per metre of shoreline basis. There were only seven empirical estimates of D_{shore} (Fig. 3*d*), and the data were collected using diverse sampling methods (mesh size, seasonality, and sampling intensity). Because of the small sample size, we fit a negative exponential model to the D_{shore} estimates (σ = 1.88 g C·m^{-1}·year^{-1}; Fig. 3*d*). From the fitted distribution, we generated 5000 D_{shore} values and 5000 corresponding lake areas (again, same number of lakes in each $\log_{10}$ size class). For simplicity, we assumed recipient lakes were circular, with a perimeter p, radius r, and area A. For each simulated lake, we calculated total flux to the lake ($D_{total} = D_{shore} \times p$; g C·year^{-1}) and the flux per square metre of lake ($D_{areal} = D_{total}/A$; g C·m^{-2}·year^{-1}). Since we assume lakes to be circular, this equation simplifies to $D_{areal} = (2D_{shore})/r$.

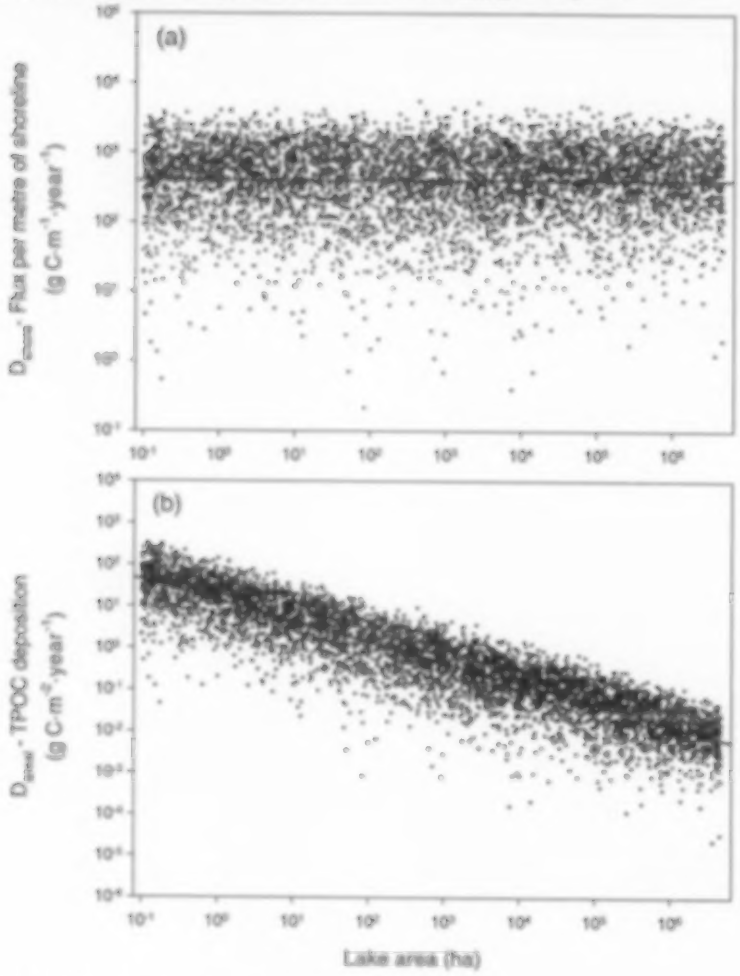
Results: land-to-lake

Airborne carbon deposition D_{shore} varied widely among lakes. In our simulation, D_{shore} was modeled to be independent of lake area (Fig. 5*a*). When expressed as per square metre of lake surface area D_{areal}, average deposition decreases as a function of lake size, as airborne TPOC deposition is diluted over a larger surface area (Fig. 5*b*). These results are intuitive — compared with large lakes, small lakes are dominated by edge and are thus subject to greater airborne inputs, all else being equal. The scatter around the trend lines (Figs. 5*a* and 5*b*) reflects among-lake variation in D_{shore}. Numerous unaccounted-for factors, such as riparian vegetation type and position relative to dominant wind direction, are likely responsible for the variation in the amount of airborne land-to-lake carbon flux.

Results: net airborne carbon flux

The overall patterns of land-to-lake airborne carbon flux

Fig. 5. Scaling of land-to-lake airborne carbon flux with lake size based on 5000 simulated lakes. (*a*) TPOC deposition per metre of shoreline: D_{shore}, g C·m^{-2}·year^{-1}; (*b*) TPOC deposition expressed on an areal basis: D_{areal}, g C·m^{-2}·year^{-1}.

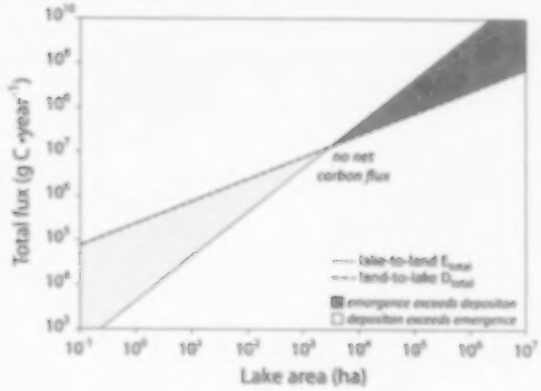


contrast with those for lake-to-land (Figs. 4 and 5). We compared how total carbon flux (E_{total} and D_{total}) scales with lake area in each direction. Both E_{total} and D_{total} increase as a function of lake area (Fig. 6). Assuming "typical" insect emergence and TPOC deposition rates, terrestrial inputs to lakes exceeds insect emergence ($D_{total} > E_{total}$) in lakes smaller than 2000 ha. For larger lakes, insect emergence exceeds aerial TPOC flux to the lake ($D_{total} < E_{total}$). For the vast majority of lakes on the planet, most of which are small (Downing et al. 2006), the airborne flux of terrestrial carbon to lakes is likely to exceed that of insect emergence. It is only in moderate-sized lakes that carbon fluxes are comparable, while in large lakes, emergence is expected to exceed terrestrial airborne inputs. Lake geometry determines the net flux across the lake–land ecotone when examined across the full range of ecosystem sizes, though there is also a high degree of variability (not shown in Fig. 6) that derives from variation in benthic production, insect emergence, and TPOC deposition rates (e.g., the scatter in Figs. 4 and 5).

Discussion

The scope of ecological research has expanded over the past decade to include the movement of nutrients, matter, and energy across habitat boundaries (Polis et al. 1997, 2004). These fluxes often have important consequences for the structure and dynamics of the recipient systems, such that the dynamics of an ecosystem are often driven by processes and fluxes emanating from an adjacent habitat or ecosystem (Polis et al. 1997, 2004). Interestingly, the importance of cross-habitat fluxes and food web linkages are surprisingly poorly known for lakes (Schindler and Scheuerell 2002). In contrast, a great body of research has now accumulated for stream ecosystems. On the one hand this makes sense; the ratio of edge to volume partially determines potential for habitat coupling (Schindler and Scheuerell 2002). Streams are generally small and are thus dominated by edge habitat, which would tend to promote coupling of the two habitats. In contrast, lakes are relatively large and are less dominated by edge habitats. On the other

Fig. 6. Comparison of total carbon flux from lake-to-land (E_{total}, solid line) and land-to-lake (D_{total}, dashed line). The point of intersection of the two lines indicates no net carbon flux. The upper right wedge (dark fill) represents the net flux from lake-to-land. The lower left wedge (light fill) represents the net flux from land-to-lake. Variability from simulations (shown in Fig. 4 and Fig. 5) is not shown to allow comparison of net fluxes.



hand, the ratio of surface area to perimeter increases with ecosystem size. As a result, the potential for emergent aquatic insect flux to land increases with ecosystem size and is generally higher for lakes compared with streams because of their larger surface area (Gratton and Vander Zanden 2009).

This study extends the earlier model of Gratton and Vander Zanden (2009) to explore the bidirectional airborne carbon fluxes across the lake–land interface. Airborne carbon fluxes to and from lakes can be expressed in several ways: on an areal basis (per square metre of lake surface area), as the flux crossing the shoreline (per metre of shoreline), or as a total carbon flux. How carbon fluxes scale with ecosystem size depends upon how values are expressed. Areal insect emergence is independent of lake area (M.J. Vander Zanden and C. Gratton, unpublished data), while flux per metre of shoreline increases with lake area. In the absence of reliable field estimates, we assumed TPOC deposition per metre of shoreline to be independent of lake area. This makes intuitive sense, though recent research indicates that shoreline deposition tended to increase with lake area in a set of 16 small (0.1–184 ha) lakes in Wisconsin (Preston 2009). Nevertheless, our model indicated that across a large gradient of lake sizes, lake-wide areal TPOC deposition decreases with lake area as a result of dilution. Comparison of total fluxes to and from lakes reveals that both increase with lake area. For small lakes, land-to-lake fluxes dominate because of the dominance of edge habitats. Lake-to-land flux increases steeply with lake area and tends to exceed land-to-lake fluxes for large lakes (>2000 ha), though there is a high degree of variability due to among-lake differences in insect emergence and TPOC deposition. For all but the largest lakes on the landscape, airborne TPOC inputs are expected to exceed carbon flux mediated by insect emergence.

Consistent with our result that emergent insect flux to shore (E_{shore}) increases with lake size, the most well-known examples of dramatic insect hatches are from large aquatic ecosystems — notable examples include chironomid midges in Lake Myvatn (Iceland; Gratton et al. 2008) and Lake Winnebago (USA; Hilsenhoff 1967), *Chaoborus* in Lake Malawi (Tanzania, Uganda, Kenya; Irvine 2000), and mayflies in the Mississippi River and Lake Erie (USA; Krieger et al. 2007). Note that our assessment generally does not include aquatic insect larvae that inhabit the pelagic zone. Most notable are dipterans of the genus *Chaoborus* (phantom midges), which make vertical migrations into the pelagic and are often not included in benthic invertebrate sampling. *Chaoborus* can comprise a large fraction of invertebrate biomass and secondary production in some lakes (Irvine 2000; Wetzel 2001).

Though we expressed cross-habitat fluxes in terms of carbon in our comparison, fluxes can also be expressed in terms of nutrients such as nitrogen or phosphorus. Nitrogen and phosphorus content in insects is high, roughly 10% and 1%, respectively (Fagan et al. 2002; Woods et al. 2004). In contrast, terrestrial particulate organic matter (TPOM) is highly heterogeneous, but is generally dominated by plant-derived material, resulting in TPOM being relatively nutrient-poor. Expressing fluxes in terms of nutrients rather than carbon increases the relative importance of the lake-to-land flux because of the higher nutrient content of emerging insects.

Our comparison considers only airborne carbon fluxes. This represents a conspicuous though potentially small fraction of the total carbon budget of a lake. A full lake carbon budget would also include fixation of dissolved inorganic carbon (DIC) by algae, inputs of terrestrial dissolved organic carbon (DOC), as well as carbon losses due to respiration, sedimentation, and the lake outlet. Considering all aspects of a lake carbon budget is beyond the scope of this paper, though we do note that other aspects of lake carbon budgets may also scale with ecosystem size.

Our goal was to consider the bidirectional nature of lake–land airborne fluxes and the potential implications for aquatic and terrestrial ecosystems. Though the available data are quite limited, we synthesize some of the existing data in an attempt to integrate these reciprocal fluxes into a quantitative framework. Consideration of aquatic ecosystem geometry was essential in understanding cross-habitat fluxes — on an overall basis, lake-to-land flux increased more rapidly as a function of ecosystem size than flux from land-to-lake, and the "outward" flux of carbon from lakes is estimated to exceed airborne carbon inputs only for relatively large lakes. The important role of ecosystem size in our comparison also highlights the sharp contrast between lakes and streams. Streams are much smaller in areal extent than lakes and should be dominated by land-to-stream linkages (Gratton and Vander Zanden 2009).

The study of ecosystems in isolation from their surrounding landscape is increasingly viewed by ecologists as inadequate (Polis et al. 2004). Though there is now broad recognition that lentic food webs are supported by a mix of benthic, pelagic, and terrestrial carbon pathways, we still know remarkably little about the trophic basis of support of higher trophic levels in lakes and how this scales with key lake attributes such as lake size and trophic status (Schindler and Scheuerell 2002; Vadeboncoeur et al. 2002; Vander Zanden and Vadeboncoeur 2002). Similarly, fluxes from lakes to

terrestrial ecosystem are poorly appreciated (Gratton and Vander Zanden 2009). While such fluxes may play a minor role in overall ecosystem carbon budgets, emergent insects are high-quality resources that are aggregated in riparian and nearshore areas. Thus, they are often an important resource for riparian consumers and ecosystems, particularly adjacent to large rivers and lakes (Gratton and Vander Zanden 2009). The broader implications of such lake-to-land fluxes are poorly explored (Finlay and Vredenburg 2007; Gratton et al. 2008; Pope et al. 2009), but the effects may exceed those reported for streams because of fundamental differences in ecosystem geometry between these ecosystems.

Acknowledgements

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CORRIGENDUM / RECTIFICATIF

Corrigendum: Mating success of alternative male phenotypes and evidence for frequency-dependent selection in Chinook salmon, *Oncorhynchus tshawytscha*

Barry A. Berejikian, Donald M. Van Doornik, Rob C. Endicott, Timothy L. Hoffnagle, Eugene P. Tezak, Megan E. Moore, and Jeffery Atkins

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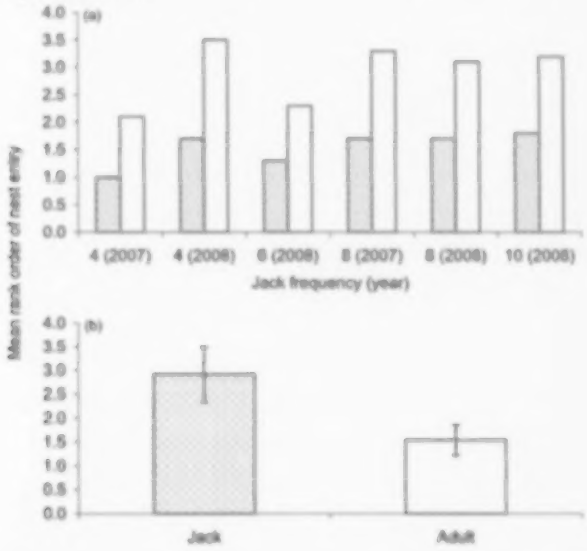
In the caption for Fig. 3 in the original article, the shading types described for panel (*a*) were mistakenly reversed. The corrected figure caption is as follows:

Fig. 3. (*a*) Average rank order of nest entry for jack (open bars) and adult (stippled bars) males. Lower ranks entered the nest sooner than higher ranks (i.e., the first male to enter the nest at the time of spawning was given a rank of 1, second to enter had a rank of 2, and so on). Panel (*b*) shows averages and 95% confidence intervals for the combined data. Note that patterns for the bars in panel (*b*) are reversed (stippled bars represent jacks and open bars represent adults).



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B.A. Berejikian,[1] **D.M. Van Doornik, R.C. Endicott, E.P. Tezak, and M.E. Moore.** NOAA Fisheries, Northwest Fisheries Science Center, Resource Enhancement and Utilization Technologies Division, Manchester Research Station, P.O. Box 130, Manchester, WA 98353, USA.
T.L. Hoffnagle. Oregon Department of Fish and Wildlife, 203 Badgley Hall, Eastern Oregon University, La Grande, OR 97850, USA.
J. Atkins. Pacific States Marine Fisheries Commission, 205 SE Spokane St., Portland, OR 97202, USA.

[1]Corresponding author (e-mail: barry.berejikian@noaa.gov).

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